Jellied Condition in the American Plaice *Hippoglossoides platessoides* (Fabricius)

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Fisheries Research Board of Canada
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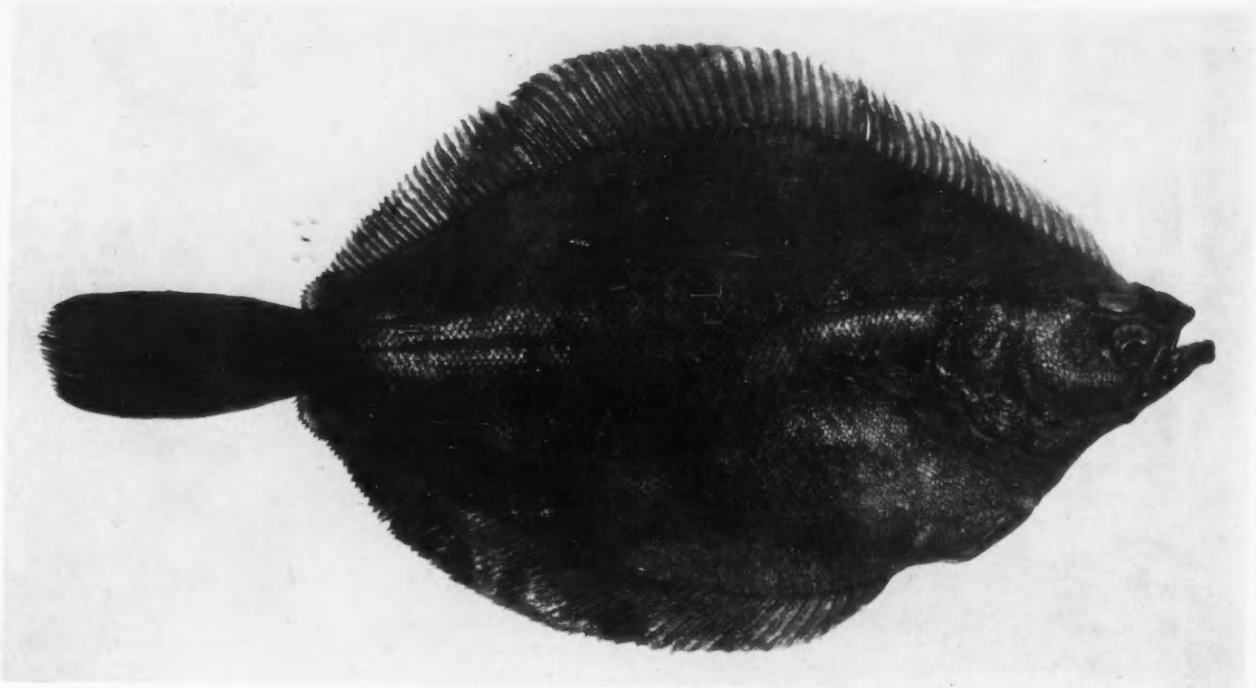

American plaice from eastern Grand Bank (female, mature, 46 cm.).

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ABSTRACT

On much of the Grand Bank a "jellied" condition in the flesh of many large American plaice renders fillets prepared from them unsuitable for the market. The jellied fillets have, on the average (in percentages of total weights), over 4% more water and over 4% less protein than normal fillets. In the jellied fillets the myosin fraction is reduced and the stroma fraction increased in terms of percentage of total nitrogen. Drip is greater in the jellied than in the normal fillets. Jellied condition is negligible in immature and small mature American plaice, but occurs increasingly at larger sizes. All very large fish are females, and most of the females above 60 cm. in length are jellied. The jellied condition in the Grand Bank area occurs throughout the year. More of the large American plaice tend to be jellied in waters between −1° and 0°C., and fewer in areas of higher bottom temperature. From the important eastern Grand Bank area, commercial catches of plaice from shallow waters (31 to 55 fath.) are composed of small fish few of which are jellied, while at increasing depths, down to 100 fath., there is a gradual increase in the size of plaice and in the percentage of jellied plaice. At 113 to 124 fath., with higher temperatures, research vessel catches indicate, for the same fish sizes, a smaller percentage of jellied fish than in the colder water at 61 to 87 fath.

Microscopic examination indicates less muscle fibre material, much greater waviness of muscle fibres, and more inter-fibre space in jellied than in normal fillet tissues. There is no evidence that sporozoan or other parasites are responsible for the typical jellied condition. The jellied fillets are edible if cut in small pieces before cooking to allow escape of water. During cooking there is considerably more shrinkage in jellied than in normal fillets. It is concluded that the jellied condition is due to protein emaciation caused by the gonads having priority in use of protein, and by the inability of many large plaice in the colder-water areas to provide for body repair and growth and for the development of sexual products. The Grand Bank jellied plaice are in a new fishing area. The largest plaice should be discarded at sea and intensive fishing will reduce the numbers of large and jellied fish.

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INTRODUCTION

The Fishery for American Plaice

In the trade these fish are not as a rule called American plaice, but rather by the names dab or flounder, and the words plaice or flounder will be commonly used in referring to the species in this paper.

The Newfoundland fishery for American plaice, *Hippoglossoides platessoides* (Fabricius), was negligible until 1948, when it began to increase. In 1946 the research vessel *Investigator II* had found plaice fairly plentiful near the south-eastern edge of the Grand Bank. In June, 1948, they were taken especially plentifully at 60 to 75 fath. by the *Investigator II* on the northeast edge of this bank, together with some cod. Soon afterward some Newfoundland trawlers began fishing for these plaice and cod. The exploratory fishing by the *Investigator II* was continued in 1949 along the whole eastern and northern part of the Grand Bank. As a result of these explorations and the commercial operations, there is now available an otter-trawl fishery primarily for American plaice on the whole eastern slope area of the Grand Bank and a fishery for plaice and cod on the northern and northwestern slopes.

On the Grand Bank the plaice fishery is mostly between 40 and 120 fath. Generally it may be said that the plaice are most abundant and largest in the path of the Labrador Current and at bottom water temperatures between $-1°$ and $+1°$C.

Figure 1 shows the catch of plaice in Subareas 3 and 4 of the International Commission for the Northwest Atlantic Fisheries (ICNAF). Subarea 3 includes the east and south coasts of Newfoundland and the Grand Bank and St. Pierre Bank areas. The amounts labelled "Canada–Total' include the catches landed in Newfoundland and in Nova Scotia. These catches are almost all by otter-trawlers. The catch was small and almost entirely by Newfoundland ships up to and including 1949. The Newfoundland fishery increased rapidly until 1952 and levelled off in 1953, while the Nova Scotian catch rapidly increased from its beginning in 1950 until it approximately equalled the Newfoundland catch during 1951–53. The United States catch has been very small and, while many millions of pounds of plaice are caught by the European vessels fishing in the area for cod for salting, these plaice are usually discarded and statistics of catch are, therefore, not available.

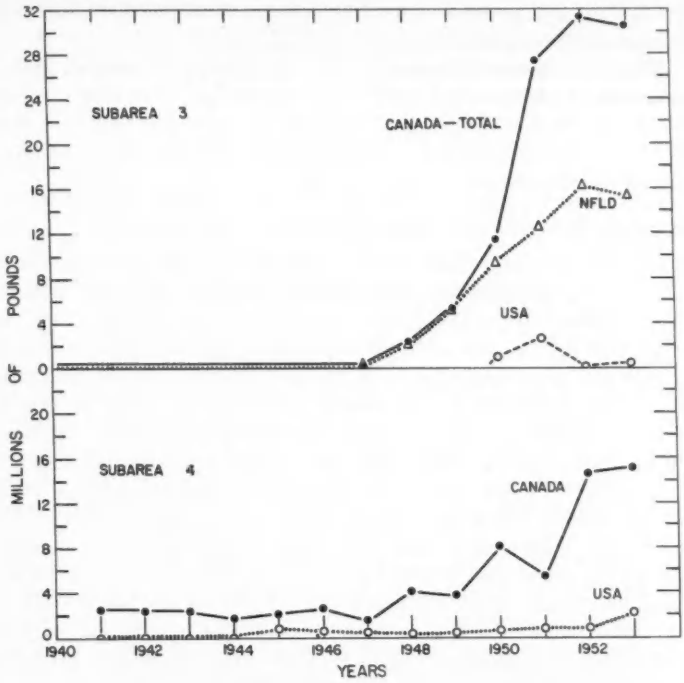

FIG. 1.—Landings of American plaice from Subareas 3 and 4 of the International Commission for the Northwest Atlantic Fisheries.

In the ICNAF Subarea 4, which includes the Bay of Fundy, the Nova Scotian area and the Gulf of St. Lawrence, the catch (Fig. 1) is landed almost entirely in the Maritime Provinces and Quebec, the catch by the United States being small and the catch by Newfoundland negligible.

Thus, Canada at the present time catches about 30 million pounds of plaice annually on the Grand Bank–St. Pierre Bank area, mostly on the eastern edge of the Grand Bank, and about 15 million pounds annually in the Bay of Fundy, Gulf of St. Lawrence and Nova Scotian area. Most of this latter amount (about 12 million pounds) comes from the Gulf of St. Lawrence, particularly from the southwestern part.

HISTORY OF THE INVESTIGATION

Early in the development of the plaice fishery it was found that an important fraction of the catch had "jellied" flesh which made it unsuitable for the market. During October, 1949, some of the Newfoundland fresh fish plants ceased taking American plaice from their trawlers because of the jelly-like condition of a high proportion of the fillets. Previously there had been some complaints from the market. This new fishery had by 1949 become quite important,

especially during the summer, autumn and early winter when haddock were not available in commercial quantities.

The Fisheries Research Board began work on these jellied flounders in November, 1949, and continued until October, 1953. During this time an effort was made to use both biologists and chemists for an investigation in which the chemical and physical conditions of the flesh were related to the life-history of the plaice and to the conditions of the fishery.

Description of Jellied and Normal Plaice

In our laboratory, fillets have been separated by sight and touch into three categories: normal, intermediate, and jellied. Fillets at the time of separation into these categories were marked only by numbers. The sex and stage of sexual maturity and, except for size of fillet, the sizes of the individual fish were unknown to the operator. Throughout the whole experiment these separations into categories were carried out by the senior author in order to standardize the separation. Sexual maturity stages were determined or checked by the senior author. The "jellied fillets" were jelly-like, quivering when touched, glossy, opalescent and not greyish. The connective tissue septa were wide and translucent, the connective tissue itself being loose and indefinite in nature. The cut face of the fillet was smooth and not ridgy, with the muscle segments or myotomes pulled away from each other. In a Waring Blendor the jellied fillet minced readily to form a thick creamy mixture which was readily poured. The fillets called jellied were such as in our opinion would be or should be rejected without question in any filleting plant. The jellied condition extended over the whole fillet, although, as is normal in flat-fishes, the flesh close to the dorsal and anal fins had the most gelatinous appearance.

The flesh of the "normal fillets" was of the usual firm consistency and was not jelly-like and not glossy or opalescent. In the distinctly higher quality fillets from the smaller fish, the cut face had a greyish tinge, definitely not white. With larger fish there was an increasing whiteness of the so-called normal fillet. The cut face of the fillet was ridgy with the myotomes standing up above the connective tissue septa. The myotomes were close together with narrow, definite and distinct connective tissue septa. In a Blendor the fillet minced much less readily than the jellied type, giving a mixture of a much firmer consistency which did not pour. The normal fillets would pass inspection in a filleting plant without question as an excellent product with regard to texture of the flesh and absence of the jellied condition.

Between the definitely jellied and the definitely normal groups it was found necessary to denote an "intermediate" stage. This intermediate group comprised fillets which, while being somewhat glossy and opalescent and intermediate in general appearance between the jellied and normal fillets described above, were yet firm to the touch, not quivering with gelatinous material, would be acceptable as an article of food, and would generally pass inspection in a filleting plant.

Obviously it is impossible by sight and touch to separate three such intergrading categories without some overlapping. This problem would be encountered also in a filleting plant or in any inspection by eye.

Length Measurement

Plaice lengths in this paper are in all cases total lengths from the tip of the snout with the mouth closed to the posterior tip of the caudal fin, and are expressed to the nearest centimetre.

CHEMICAL ANALYSIS OF PLAICE

Methods

The analysis of American plaice fillets for moisture, protein and fat content was begun in December, 1949. The results given in the following tables date from January, 1950, to August, 1952. The fillets were first numbered with the fish tag number, then divided into three categories: normal, intermediate and jellied. From these categories, samples picked by random choice of numbers were used in the analyses. Each pair of fillets was blended in a Waring Blendor before sampling.

The percentage moisture was determined in triplicate and at times in quadruplicate, using 10- to 20-g. samples in small aluminium dishes and drying to relatively constant weight usually in three periods of 16, 6 and 16 hours in a hot air oven at 100 to 110°C. At the end of each period, after cooling in a desiccator, the dried samples were weighed. Beyond the 38-hour drying period, in test examples, further drying for two 16-hour periods resulted in a further total loss of less than 0.1% of water. The dried material was then finely ground in a mortar and placed in stoppered containers. The fat was determined by continuous extraction overnight in a Bailey-Walker extractor using absolute ether as solvent. Total nitrogen content was determined by the macrokjeldahl method, using anhydrous sodium or potassium sulphate, copper sulphate and concentrated sulphuric acid as the digestion mixture. The protein results given are the percentages of total nitrogen multiplied by the factor 6.25. The fat and protein percentages were then converted from the dry to the original wet basis.

Frequency Distribution of Water, Fat and Protein Values

One important difference between normal and jellied fish is an increase of about 4% in water content from the normal to the jellied fillets (Tables I and V).

Table I.—Average percentage fat and protein of normal (N), intermediate (I) and jellied (J) American plaice fillets at each moisture range.

Range of percentage moisture	Number of fish			Percentage fat			Percentage protein			Standard deviation percentage fat			Fat "t" value between means		
	N	I	J	N	I	J	N	I	J	N	I	J	N and I	N and J	I and J
77.01–78.00	1	...	...	4.82	...	...	15.51	...	...	...	...	...	...	...	...
78.01–79.00	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
79.01–80.00	1	...	2	0.18	...	6.64	17.89	...	12.61	...	...	...	...	...	...
80.01–81.00	17	...	...	0.80	...	...	16.89	...	...	0.699	...	...	...	...	...
81.01–82.00	22	2	...	0.72	2.11	...	16.24	15.05	...	0.568	...	...	...	...	...
82.01–83.00	53	6	1	0.53	1.36	1.79	15.54	14.50	13.91	0.510	0.562	...	3.74	...	...
83.01–84.00	50	14	3	0.44	1.35	1.19	14.75	13.60	13.66	0.428	0.874	0.810	5.45	2.82	...
84.01–85.00	17	35	10	0.35	0.77	1.60	13.75	13.40	12.21	0.188	0.411	0.953	4.00	5.31	4.07
85.01–86.00	4	31	17	0.34	0.67	1.00	13.14	12.47	12.02	0.247	0.544	0.783	1.19	1.64	1.72
86.01–87.00	...	20	27	...	0.35	0.79	...	11.80	11.34	...	0.164	0.691	...	...	2.78
87.01–88.00	...	4	24	...	0.40	0.59	...	10.76	10.45	...	0.189	0.450	...	...	0.82
88.01–89.00	...	1	9	...	0.51	0.26	...	9.61	9.97	...	...	0.174	...	...	...
89.01–90.00	...	...	11	...	...	0.66	...	...	8.42	...	...	0.569	...	...	...
90.01–91.00	...	...	2	...	...	0.36	...	...	7.39	...	...	...	...	...	...
91.01–92.00	...	...	1	...	...	0.01	...	...	7.42	...	...	...	...	...	...
92.01–93.00	...	...	1	...	...	0.25	...	...	6.31	...	...	...	...	...	...
Total	165	113	108												
			Average	0.55	0.78	0.90	15.31	12.85	10.87						

There is little difference in the (usually small) fat content. The increase is made at the expense of protein, which decreases by 29% of its own normal bulk. Thus, the gain in moisture content would appear to be the result of a replacement of the lost protein.

In Tables II and III, quite naturally the results for intermediate fillets show

TABLE II.—Percentage frequencies of normal (N), intermediate (I) and jellied (J) plaice fillets in various moisture and moisture plus fat ranges.

Frequency range in percentage	Percentage of totals					
	Moisture			Moisture + fat		
	N	I	J	N	I	J
77.01–78.00	0.6	...	...	...	...	...
78.01–79.00	...	...	...	...	...	...
79.01–80.00	0.6	...	1.9	...	...	...
80.01–81.00	10.3	...	...	2.4	...	...
81.01–82.00	13.3	1.8	...	12.1	...	...
82.01–83.00	32.1	5.3	0.9	24.9	...	...
83.01–84.00	30.3	12.4	2.8	35.2	6.2	0.9
84.01–85.00	10.3	31.0	9.3	18.2	18.6	1.9
85.01–86.00	2.4	27.4	15.7	6.7	33.6	9.3
86.01–87.00	...	17.7	25.0	0.6	27.4	25.0
87.01–88.00	...	3.5	22.2	...	10.6	23.1
88.01–89.00	...	0.9	8.3	...	3.5	20.4
89.01–90.00	...	...	10.2	...	...	9.3
90.01–91.00	...	...	1.9	...	...	5.6
91.01–92.00	...	...	0.9	...	...	3.7
92.01–93.00	...	...	0.9	...	...	0.9
Total fish	165	113	108	165	113	108

TABLE III.—Percentage frequencies of normal (N), intermediate (I) and jellied (J) plaice fillets in various fat and protein ranges.

Frequency range in percentage fat	Percentage of totals			Frequency range in percentage protein	Percentage of totals		
	N	I	J		N	I	J
0.04–0.20	26.1	7.1	8.3	6.01– 7.00	...	...	1.9
0.21–0.40	27.3	21.2	31.5	7.01– 8.00	...	...	3.7
0.41–0.60	18.2	22.1	14.8	8.01– 9.00	...	...	8.3
0.61–0.80	8.5	17.7	10.2	9.01–10.00	...	0.9	13.0
0.81–1.00	6.7	7.1	7.4	10.01–11.00	...	3.5	23.1
1.01–1.20	3.0	5.3	3.7	11.01–12.00	...	15.0	20.4
1.21–1.40	4.2	7.1	3.7	12.01–13.00	1.8	38.1	24.1
1.41–1.60	1.2	3.5	5.6	13.01–14.00	9.7	29.2	4.6
1.61–1.80	0.6	1.8	4.6	14.01–15.00	24.2	10.6	0.9
1.81–2.00	0.6	1.8	1.9	15.01–16.00	37.0	2.7	...
2.01–2.20	1.2	0.9	0.9	16.01–17.00	21.2	...	...
2.21–2.40	...	0.9	1.9	17.01–18.00	6.1	...	...
2.41–2.60	1.2	1.8	0.9				
2.81–3.00	0.6	...	1.9				
3.01–3.20	...	1.8	...				
4.01–4.20	...	...	0.9				
4.81–5.00	0.6	...	...				
6.41–6.60	...	...	0.9				
6.61–6.80	...	...	0.9				
Total fish	165	113	108		165	113	108

some overlapping with those for both normal and jellied fillets, but the frequencies for normal and jellied fillets are practically distinct. The fat frequencies show no very distinct difference between normal and jellied samples. However, because individual fat values may vary considerably, the combined moisture and fat results give a better basis for comparison, e.g. a jellied fillet with a very high fat content will usually have a moisture content in what is usually considered the moisture range of normal flounder. Table I shows the moisture–fat relationship. In each category, a decrease in the percentage of fat is shown with increasing moisture content, while the jellied and intermediate fillets have a higher fat content than the normal at the same moisture range. This further bears out the fact that the moisture plus fat content is a better criterion than moisture content alone for separation of the three categories.

Variation with Size of Plaice

Table IV shows that there is a gradual increase in both moisture and fat content with increasing length of fish, and a corresponding decrease in protein.

Table IV.—Average percentages* of moisture, fat, moisture plus fat, and protein in the fillets at various size ranges of normal (N), intermediate (I) and jellied (J) plaice. January, 1950–March, 1952.

		Length range, cm.							Total No. fish
		26–33	34–41	42–47	48–53	54–61	62–71	74	
Number of fish	N	22	38	51	40	9	2	...	162
	I	1	4	17	38	33	20	...	113
	J	...	...	11	18	54	22	1	106
Average percentage moisture	N	82.19	82.10	82.92	83.06	83.96	83.45	...	
	I	(81.70)	83.97	84.34	85.11	85.26	85.09	...	
	J	...	...	86.86	86.16	86.93	87.22	(89.26)	
Average percentage fat	N	0.30	0.35	0.58	0.79	0.68	1.54	...	
	I	(1.61)	0.96	0.58	0.66	0.70	1.17	...	
	J	...	...	0.34	0.72	0.86	1.10	(1.87)	
Average percentage moisture+fat	N	82.49	82.45	83.49	83.85	84.64	84.98	...	
	I	(83.31)	84.93	84.92	85.77	85.97	86.26	...	
	J	...	...	87.20	86.88	87.79	88.33	(91.13)	
Average percentage protein	N	16.10	16.09	15.08	14.72	14.01	13.65	...	
	I	(15.30)	13.36	13.53	12.87	12.59	12.41	...	
	J	...	...	11.38	11.76	10.79	10.26	(7.41)	

*Values in parentheses indicate averages from one fish.

This is reflected in the appearance of the fillets. The firmest of the normal fillets have a greyish colour and are restricted to the smaller sizes of fish. With increasing size, the fillets become whiter and more glossy in appearance.

Table V indicates that there are some significant differences in the moisture, fat and protein content of fillets of the same condition at different times in the year. However, in different periods of the year fishing is carried on in different areas of the Grand Bank. On the eastern slope, fishing is towards the south in winter and early spring and with a northward tendency in summer. Many of

TABLE V.—Quarterly averages of moisture, fat, moisture plus fat, and protein, in fillets of normal (N), intermediate (I) and jellied (J) plaice. January, 1950–March, 1952.

	Time of year	Number of fish			Average fish size (cm.)			Average percentages			Standard deviation		
		N	I	J	N	I	J	N	I	J	N	I	J
Moisture	Jan.–Mar.	26	18	15	46	60	61	83.36	85.40	87.39	0.98	0.83	2.08
	April–June	38	27	32	42	52	56	82.84	84.93	87.45	0.98	0.92	1.57
	July–Sept.	58	44	34	44	53	57	82.79	85.17	86.78	1.39	1.56	1.78
	Oct.–Dec.	27	22	20	43	52	57	82.39	84.31	86.14	1.08	1.30	1.77
	All year	149	111	101	43	54	57	82.83	84.98	86.96	1.20	1.31	1.80
Fat	Jan.–Mar.	26	18	15	46	60	61	0.26	0.53	1.04	0.18	0.40	1.09
	April–June	38	27	32	42	52	56	0.47	0.70	0.31	0.49	0.41	0.17
	July–Sept.	58	44	34	44	53	57	0.79	0.94	1.08	0.80	0.78	0.74
	Oct.–Dec.	27	22	20	43	52	57	0.60	0.74	0.92	0.49	0.46	0.51
	All year	149	111	101	43	54	57	0.58	0.78	0.80	0.62	0.60	0.72
Moisture + fat	Jan.–Mar.	26	18	15	46	60	61	83.62	85.93	88.43	0.98	0.71	1.86
	April–June	38	27	32	42	52	56	83.32	85.63	87.77	0.99	0.77	1.53
	July–Sept.	58	44	34	44	53	57	83.57	86.12	87.86	1.10	1.19	1.54
	Oct.–Dec.	27	22	20	43	52	57	82.98	85.05	87.06	1.05	1.07	1.60
	All year	149	111	101	43	54	57	83.41	85.75	87.76	1.06	1.09	1.63
Protein	Jan.–Mar.	26	18	15	46	60	61	15.07	12.75	10.10	0.96	0.90	1.79
	April–June	38	27	32	42	52	56	15.43	13.03	10.97	0.98	0.76	1.58
	July–Sept.	58	44	34	44	53	57	14.87	12.43	10.64	1.04	1.25	1.52
	Oct.–Dec.	27	22	20	43	52	57	15.55	13.48	11.46	1.08	1.02	1.52
	All year	149	111	101	43	54	57	15.17	12.85	10.83	1.05	1.11	1.61

the samples of flounder analysed came from commercial catches so that there were not enough samples from the same area at all seasons to permit any definite conclusion as to whether seasonal differences in chemical constitution occur within the same area.

FRACTIONATION OF PROTEIN

METHODS

In 1953 an experiment was made to determine whether, in the loss of protein in the jellied condition, the composition of the remaining protein was changed. This was done by fractionating the proteins according to the methods used by Dyer, French and Snow (1950). The samples were sliced from frozen fillets and blended in a Waring Blendor with a semi-frozen solution of 5% sodium chloride containing a small amount of sodium bicarbonate. After centrifuging, samples were taken for determination of soluble protein nitrogen and non-protein nitrogen and myosin. The stroma nitrogen was determined from the residue, after washing with dilute acid and alkali. The total soluble protein nitrogen was determined using a biuret reagent and an Evelyn colorimeter, and the myosin was determined by the same method after overnight precipitation from the salt solution by dilution with water. The non-protein nitrogen was determined by both micro- and macro-kjeldahl methods from the salt solution and from a weighed sample after precipitation of the protein with a solution of trichloroacetic acid. Percentages of moisture, total nitrogen, and fat were determined by the same methods as given previously for the regular analysis of flounder.

Differences Between Proteins of Normal and Jellied Fish

The average results from protein fractionation of 32 samples of normal and 34 of jellied plaice are given in Table VI. In all protein fractions compared there were highly significant differences between the normal and the jellied fillets. A great difference was shown in the myosin fraction of the soluble protein, which

Table VI.—Results of protein fractionation in normal (N) and jellied (J) plaice fillets.

	Normal	Jellied	Number of samples		Standard deviation		"t" value	Probability
			N	J	N	J		
Range of total percentage nitrogen	2.85–2.31	2.10–1.14	—	—	—	—	—	—
Average total percentage nitrogen	2.58	1.60	32	34	0.12	0.26	19.41	0.001
Ratio stroma to myosin × 100	8.68	13.3	31	33	2.42	4.41	5.19	0.001
As percentage total nitrogen:								
Non-protein nitrogen	14.0	15.7	32	34	0.68	1.51	6.15	0.001
Protein nitrogen	86.1	84.3	32	34	0.68	1.51	6.19	0.001
As percentage of total protein nitrogen:								
Total soluble protein nitrogen	92.8	88.5	32	34	2.94	4.97	4.24	0.001
Myosin nitrogen	77.2	66.2	32	34	4.70	9.85	5.73	0.001
Non-myosin nitrogen	15.5	22.2	32	34	2.84	8.75	4.13	0.001
Stroma nitrogen	6.6	8.7	31	33	1.61	2.24	4.27	0.001
Percentage moisture	82.5	88.2	32	33	0.73	1.84	16.18	0.001
Percentage fat	0.77	1.02	26	27	0.31	0.85	1.42	0.2

averaged 77.2% of the total protein nitrogen for normal and only 66.2% for jellied fish. The non-protein nitrogen percentage of the total nitrogen, and the stroma nitrogen percentage of the total protein nitrogen, were both higher in the jellied samples. Thus most of the protein loss can be accounted for by reduction of the amount of myosin (muscle fibre material) while the stroma, which includes the connective tissue, has not been reduced to as great a degree.

CONDITION OF PLAICE ON CAPTURE AND ON LANDING

Since there was a possibility that the jellied condition might originate between the times of catching and landing, experiments to check this point were carried out in 1952 and 1953. Observers were placed on St. John's trawlers and they noted the plaice quality immediately on capture. The jellied condition could be distinguished in fish as they were removed from the net, so presumably it was present in the living fish. It was not so readily determined at this time, however, as later. To check this further, plaice were selected immediately after catching, and half of the upper fillet (alternately anterior and posterior) was

numbered and then skinned and placed in a bottle sealed with a rubber gasket and buried in ice after being classified as either normal or jellied. The fish with the remaining skin-on portion of the fillet was tagged and stored in ice in a fish box.

Although the bottled fillet was not frozen, a certain amount of drip (free liquid) was found in the bottle on landing 2 to 5 days later. This drip averaged 2.1% (by weight) for normal and 14.4% for jellied fillets. Within two ranges of normal fillets and two of jellied, the average drip increased with increasing moisture content (Table VII). After measurement, the drip was added to the

TABLE VII.—Comparison of sea and shore condition of jellied and normal fillets of American plaice.

	Normal		Jellied		Total normal	Total jellied
Moisture range percentages, sea samples						
Lower limit	79.27	81.24	84.76	91.45	79.27	84.76
Upper limit	81.18	84.73	90.42	96.18	84.73	96.18
Number of pairs of samples	11	10	9	8	21	17
Av. percentage moisture, sea samples	80.53	82.29	88.68	93.47	81.37	90.93
Av. percentage moisture, lab samples	82.07	82.84	87.88	92.61	82.44	90.11
Standard deviation between paired samples	0.38	0.05	1.26	0.75	0.66	1.02
"t" value	13.48	3.59	1.91	3.25	7.43	3.37
Probability	0.001	0.01	0.1	0.02	0.001	0.01
Av. percentage moisture + fat, (sea)	81.54	83.08	89.46	93.73	82.27	91.46
Av. percentage moisture + fat, (lab)	82.57	83.36	88.57	92.97	82.95	90.64
Standard deviation in percentage moisture + fat differences	0.59	0.76	1.42	0.81	0.76	1.14
"t" value (between paired samples)	5.79	1.17	1.89	2.65	4.04	3.01
Probability	0.001	0.3	0.1	0.3	0.001	0.01
Av. percentage protein, sea samples	17.14	15.84	9.23	5.11	16.52	7.29
Av. percentage protein, lab samples	16.40	15.43	10.23	5.85	15.94	8.17
Range of drip, sea samples						
Lower limit	0	0	4.8	14.2	0	4.8
Upper limit	7.3	7.3	18.7	23.1	7.3	23.1
Av. percentage drip (by weight), sea samples	1.4	2.8	10.3	18.9	2.1	14.4
Standard deviation percentage drip	2.44	2.85	4.50	3.18	2.68	5.82
"t" value	1.24		4.49		8.61	
Probability	0.2		0.001		0.001	

bottled fillet and the total taken for analysis. (For convenience, the bottled fillet will be known as the "sea sample" and the half remaining on the fish as the "laboratory sample").

The classification of the laboratory samples in nearly all cases corresponded with the observed condition at sea. The only exceptions were three intermediates, which on shore were classified as two jellied and one normal sample.

The analyses (Table VII) bear out the conclusion that the essential qualities causing the jellied condition are present in the living fish. In all samples designated at sea as jellied, the moisture content of the sea samples was well within

the usual jellied range. This experiment also showed the sea samples of jellied fillets to have a higher moisture content than the corresponding laboratory samples, whereas in the normals the reverse was true. There would seem to be, therefore, a tendency for normal fish to gain a little water during the storage in ice and for the jellied ones to lose some water during this period. To rule out any possibility of high varying fat contents affecting the figures, a comparison was also made between the moisture plus fat percentages and this showed the same tendency. In any case, the percentage protein varied inversely with the moisture, as was true of the previous analyses.

The most extreme jellied condition was found during this experiment. One of the sea-sample fillets had the following composition: 96.18% water, 0.06% fat and 2.83% protein.

It is interesting to note also that the percentage fat in the anterior half of the top fillet was greater than that in the posterior half in all cases but four, and in most cases considerably so. The collectors of the samples at sea found it more difficult to distinguish the jellied from the normal condition in the posterior parts of the fillets.

It is possible, as some plant operators believe, that visual evidence of the jellied condition, judged by flabbiness of tissue, increases progressively during the period in ice. The jellied fillets in ice in boxes for about 2 to 6 days in our experiment lost almost 1% moisture. Under the conditions of great pressure in ice in the trawler hold they would doubtless lose considerably more, for, as Cutting (1951) has shown, the weight loss by the fish (including both water and soluble protein) increases considerably with the length of the period under ice in the pounds. On the average, plaice are more jellied as the percentage of water is higher and the percentage of protein lower. At the same water and protein contents, however, some plaice fillets are normal and some are jellied. Thus, some of the jellied condition is due to the physical as well as to the chemical condition. The shipboard ice-storage effects of reducing water and hence increasing relative protein content in jellied fillets actually makes them more "normal" from a chemical point of view, though doubtless more "jellied" from a physical viewpoint.

DRIP IN NORMAL AND IN JELLIED QUICK-FROZEN PLAICE FILLETS

A comparison has been made between the amount of liquid separating on thawing (the drip) from ordinary commercial-pack plaice fillets and that separating from fillets discarded commercially as jellied. The fillets were taken from a local plant. The commercial-pack fillets, already frozen, were in cartons which were waxed or covered either with wax paper or cellophane and the flesh itself was wrapped in cellophane inside the carton. The time of storage probably ranged from a few weeks to several months. Selection of jellied samples of fresh plaice fillets was at first done by an employee of the plant. These were then quick-frozen and held in storage for about 24 hours. Later the jellied samples (as discarded in the plant) were taken randomly by Research Board personnel and treated similarly. Since the averages from these two methods of random

selection showed no significant differences, the information from both selection methods has been combined in Table VIII.

The average drip in the various moisture-range groups of normal fillets ranged from about 16 to 21% as compared with about 23 to 29% in the jellied fillet ranges. From the similarity of the moisture range distribution in the commercial pack to that of the normal plus some of the intermediate type shown in Table I, it is unlikely that drying out in storage has affected these results. In the same moisture ranges and with approximately the same protein and fat content, there is a much greater percentage drip in the fillets discarded as

TABLE VIII.—Average percentage* moisture, protein, fat and drip in normal commercial-pack fillets (NCP) and jellied commercial discards (JCD).

Frequency range for moisture, as percentage	Number of fish		Average percentage moisture		Average percentage protein		Average percentage fat	
	NCP	JCD	NCP	JCD	NCP	JCD	NCP	JCD
81.99–83.00	5	...	82.6	...	15.6	...	1.09	...
83.01–84.00	8	1	83.6	(83.1)	14.4	(14.8)	0.85	(1.29)
84.01–85.50	12	5	84.7	84.6	14.0	13.4	0.60	1.28
85.51–87.00	5	10	86.1	86.4	12.4	11.6	0.54	1.07
87.01–88.50	...	16	...	87.7	...	10.7	...	0.88
88.51–91.55	...	12	...	89.5	...	9.4	...	0.36

	Range percentage drip		Average percentage drip		Ratio $\frac{\text{percentage drip}}{\text{percentage moisture}}$	
	NCP	JCD	NCP	JCD	NCP	JCD
81.99–83.00	9.5–18.1	...	15.5	...	18.8	...
83.01–84.00	13.9–23.0	(23.1)	18.9	(23.1)	22.6	(27.8)
84.01–85.50	13.2–27.5	20.6–34.0	18.3	26.1	21.6	30.9
85.51–87.00	16.2–24.8	23.2–30.2	20.6	27.0	23.9	31.3
87.01–88.50	...	23.3–35.3	...	29.3	...	33.4
88.51–91.55	...	17.1–33.6	...	27.8	...	31.1

*Values in parentheses represent averages from one sample.

jellied. There must, therefore, be some factor apart from the moisture content which allows greater drip in the jellied flesh.

The muscle tissue in the jellied fillets has shrunken and, compared with normal fillets, the inter-cellular areas are expanded. Thus, we suspect that the jellied fillets contain a larger fraction of their moisture in the form of lymph in these expanded inter-cellular spaces. This extra-cellular moisture, being more loosely held, would presumably be more available as drip. This experiment shows the difference between the drip from the normal commercial pack and from recently discarded jellied fillets. For more accurate comparison of the higher percentage of drip from jellied fillets, in the same moisture range as the commercial-pack fillets, the experiment should be on normal and on jellied fillets from the same catch. The storage time for each would then be the same. There are at least two possible explanations for this extra drip in the same moisture range. Firstly, the jellied flounder, even in the same moisture range, could have a different chemical or physical constitution from the one accepted as normal.

Secondly, a physical change may have occurred during shipboard storage in ice, producing a more jellied appearance and a decreased ability to retain water.

RELATIONSHIP OF FILLET CONDITION TO SIZE, SEX AND SEXUAL MATURITY

Table IX shows the relationship of size, sex and condition of sexual maturity of plaice to the condition of the fillet. This table includes the plaice from areas 7, 8, 8A, 9 and 10 of Fig. 2, that is, the northwestern, northern and eastern sections of the Grand Bank. It is in these areas that most of the commercial fishing for plaice in Newfoundland waters is carried out. The plaice are large

TABLE IX.—Percentages of normal (N), intermediate (I) and jellied (J) immature (Imm) and mature (Mat) male and female and total plaice at various total length ranges from the northern and eastern Grand Bank; areas 7, 8, 8A, 9 and 10 of Fig. 2.

Length range	Numbers				Percentages																				
					Males						Females						Totals								
	Males		Females		Imm			Mat			Imm			Mat			Males			Females			Total		
	Imm	Mat	Imm	Mat	N	I	J	N	I	J	N	I	J	N	I	J	N	I	J	N	I	J	N	I	J
cm.																									
16–19	5	0	9	0	100	0	0	...	...	...	100	0	0	...	...	...	100	0	0	100	0	0	100	0	0
20–23	9	2	17	0	100	0	0	100	0	0	100	0	0	...	...	...	100	0	0	100	0	0	100	0	0
24–27	17	12	17	0	100	0	0	100	0	0	100	0	0	...	...	...	100	0	0	100	0	0	100	0	0
28–31	18	24	34	0	100	0	0	100	0	0	97	0	3	...	...	...	100	0	0	97	0	3	99	0	1
32–35	12	40	46	0	100	0	0	100	0	0	100	0	0	...	...	...	100	0	0	100	0	0	100	0	0
36–39	3	89	54	7	100	0	0	99	1	0	98	2	0	86	14	0	99	1	0	97	3	0	98	2	0
40–43	1	162	32	31	100	0	0	93	6	1	100	0	0	94	6	0	93	6	1	97	3	0	94	5	0.4
44–47	0	364	19	70	...	...	...	70	23	6	95	0	5	81	11	7	70	23	6	84	9	7	73	21	6
48–51	0	134	3	246	...	...	...	44	36	20	67	33	0	65	28	7	44	36	20	65	28	7	58	31	11
52–55	0	7	0	350	...	...	...	14	57	29	...	...	...	40	37	23	14	57	29	40	37	23	39	38	23
56–59	0	1	1	284	...	...	...	0	0	100	0	0	100	25	40	35	0	0	100	25	40	35	24	40	36
60–63	0	1	0	177	...	...	...	0	100	0	...	...	...	15	28	58	0	100	0	15	28	58	15	28	57
64–67	...	...	0	78	...	...	...	...	...	...	...	...	...	9	26	65	...	...	...	9	26	65	9	26	65
68–77	...	...	0	30	...	...	...	...	...	...	...	...	...	7	13	80	...	...	...	7	13	80	7	13	80
Total	65	836	232	1,273	100	0	0	76	18	6	98	1	1	39	31	30	77	17	6	48	27	25	59	23	18

and show throughout the year a considerable proportion of jellied individuals (Fig. 3).

In both sexes, the numbers of immature fish in the jellied condition were negligible. All of 65 immature males were normal and of 232 immature females only 3 were jellied and one was intermediate. The remainder were normal. Of the 3 jellied, one was so small as to be definitely immature; however, the jellied female at 56–59 cm. and even the one at 44–47 cm. could possibly have been mature fish incorrectly classified.

Of the mature males only 6% were jellied and 76% were normal, while of the mature female plaice 30% were jellied and only 39% normal.

Males became jellied only at the larger sizes. At the peak size of 44–47 cm. only 6% of the males were jellied. After this size, however, the percentage of normal males declined rapidly. Twenty percent were jellied at 48–51 cm. and above this size range more were jellied but the numbers were too small to afford adequate comparison.

The mature females from the areas considered showed no jellied condition below 44–47 cm. At these lengths and also at 48–51 cm., 7% were jellied. From 52–55 cm. in length, with 23% jellied and 40% normal, the percentage jellied increased rapidly until at 68–77 cm. 80% were jellied and only 7% were normal.

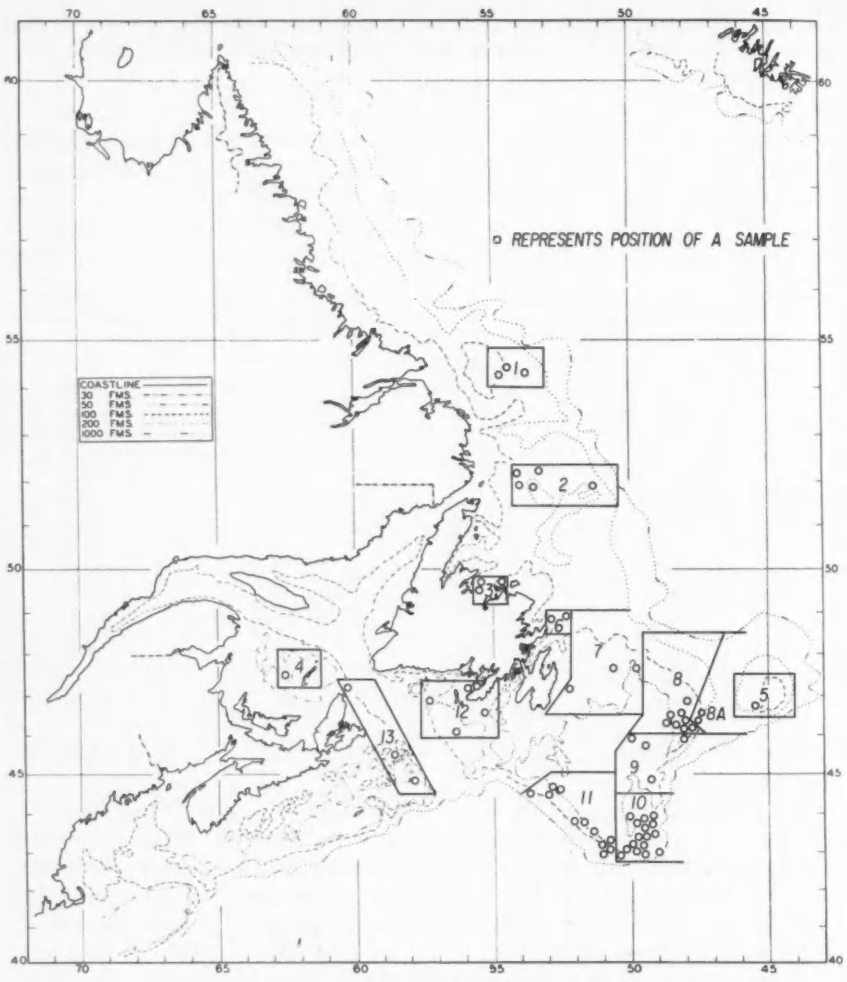

FIG. 2.—Location of plaice samples examined for fillet condition.

Male plaice mature at a much smaller size than females and do not reach the large size attained by the females (Table IX). Thus, almost all the fish of 52 cm. and of greater length, in which the jellied condition is so significant and the fish weight comparatively large, are females. It is indicated from otolith readings that these large females are often over twenty and some of the largest close to forty years of age. The jellied condition becomes significant at a smaller size in the males but in the commercial catch the problem with jellied plaice is chiefly related to the large, and especially to the very large, female fish.

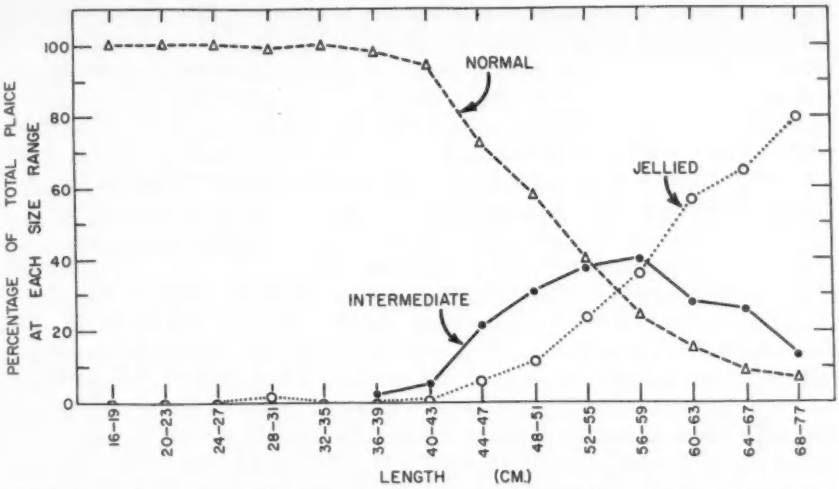

FIG. 3.—Percentages of plaice in the normal, intermediate, and jellied condition. (These plaice are from the northwestern, northern and eastern Grand Bank, areas 7, 8, 8A, 9, and 10 of Fig. 2.)

WEIGHTS OF NORMAL, INTERMEDIATE AND JELLIED PLAICE

Table X shows the differences in average gutted and gilled weights of normal, intermediate and jellied plaice at the same sizes. These weights were obtained on shore from plaice caught by the *Investigator II* and by local trawlers. The data from both sexes are combined here, since the weights of male and female plaice making up the totals have been compared without discovering any

TABLE X.—Average gutted and gilled weights of normal (N), intermediate (I) and jellied (J) plaice of length 46 cm. and over from all areas.

	Number of fish			Average weight			Per cent of weight of normal	
Length	N	I	J	N	I	J	I	J
cm.				*pounds*	*pounds*	*pounds*		
46	83	25	6	1.67	1.69	1.64	101.2	98.2
47	70	19	9	1.82	1.74	1.75	95.6	96.1
48	67	24	9	1.90	1.85	1.81	97.4	95.3
49	64	27	11	2.05	1.95	2.01	95.1	98.0
50	50	14	2	2.13	2.08	1.94	97.7	91.1
51	59	28	7	2.22	2.24	2.05	100.9	92.3
52	45	23	17	2.40	2.29	2.45	95.4	102.1
53	44	33	12	2.50	2.41	2.59	96.4	103.6
54	21	22	12	2.58	2.61	2.40	101.2	93.0
55	16	26	20	2.85	2.70	2.73	94.7	95.8
56	20	20	19	3.01	2.95	2.97	98.0	98.7
57	15	26	21	2.98	3.01	3.06	101.0	102.7
58	10	20	19	3.24	3.27	3.18	100.9	98.1
59	10	10	13	3.50	3.30	3.35	94.3	95.7
60	7	11	18	3.78	3.64	3.72	96.3	98.4
61	12	20	34	3.92	3.89	3.97	99.2	101.3
62	9	11	24	4.07	4.08	4.19	100.2	103.0
Total	602	359	253		Average of percentages		98.0	97.9

apparent consistent and significant differences at the same size. There is a good possibility that normal plaice may be about 2% heavier than intermediate and jellied plaice of the same length. Since the fish were measured only to the nearest centimetre, and since with increasing length the percentage of jellied plaice is increasing and that of normal plaice decreasing, the jellied fish would tend to be relatively more numerous in the upper part of each centimetre range and everything else being equal would thus have a tendency to be heavier than the normal plaice in the same centimetre length range. Since, however, the jellied fish are on the average lighter than normal fish the probability is increased that the normal plaice shown in Table X are slightly heavier at the same length than the intermediate and the jellied fish. However, as shown elsewhere in this paper, the flesh of jellied fish tends to lose water slightly while the fish are in ice, and that of normal fish to add water. Thus, even the slight differences between normal and jellied fish shown in landed weights in Table X may not exist in the living fish. At each length there is much overlap in weight between jellied and normal fish. Differences in weight, therefore, cannot be used to separate normal from jellied plaice. Several large intermediate and jellied plaice were, however, very emaciated, a 54-cm. male weighing 1.1 pounds less than normal, and a 61-cm. male 1.9 pounds less than normal plaice at that size. Only rarely does a male plaice attain a size of 61 cm.

SEASONAL OCCURRENCE OF JELLIED CONDITION IN PLAICE FROM EASTERN GRAND BANK

Table XI indicates the quality of plaice fillets by 3-month periods throughout the year. The area from which the fish were obtained was the eastern edge of

TABLE XI.—Seasonal (quarterly) percentages of normal, intermediate, and jellied plaice from the eastern Grand Bank. (Includes all 1950–53 fish from areas 8, 9, 10 of Fig. 2, omitting the two deep-water samples from 8A.)

	Length range, cm.								
	18–23	24–29	30–35	36–41	42–47	48–53	54–59	60–65	66–77
Number of fish examined									
Jan.–March	...	...	...	1	10	17	11	14	11
April–June	3	18	31	73	185	203	163	42	6
July–Sept.	...	16	23	40	158	154	111	78	7
Oct.–Dec.	4	15	28	56	178	143	62	60	23
Percentage normal									
Jan.–March	...	...	...	100	70	65	36	14	27
April–June	100	100	100	97	76	43	22	10	0
July–Sept.	...	94	100	97	77	53	15	13	0
Oct.–Dec.	100	100	100	100	79	56	35	15	0
Percentage intermediate									
Jan.–March	...	...	...	0	20	35	45	57	45
April–June	0	0	0	3	19	39	44	24	33
July–Sept.	...	0	0	0	20	34	46	27	14
Oct.–Dec.	0	0	0	0	15	30	35	32	13
Percentage jellied									
Jan.–March	...	...	...	0	10	0	18	29	27
April–June	0	0	0	0	5	17	34	67	67
July–Sept.	...	6	0	3	3	13	39	60	86
Oct.–Dec.	0	0	0	0	6	14	29	53	87

the Grand Bank, areas 8, 9, 10 in Fig. 2. Only from the eastern side of the Grand Bank is year-round information available and here, also, the greater part of the plaice fishery in the Newfoundland region is carried on.

Jellied plaice are present in numbers throughout the year. In all but one size category, the percentage of jellied fish was least in January–March. Although relatively few fish were examined in any one size range, the combined effect appears significant. During the remainder of the year there is little difference. October–December has less of jellied plus intermediate types than do the April–June and July–September periods (Fig. 4).

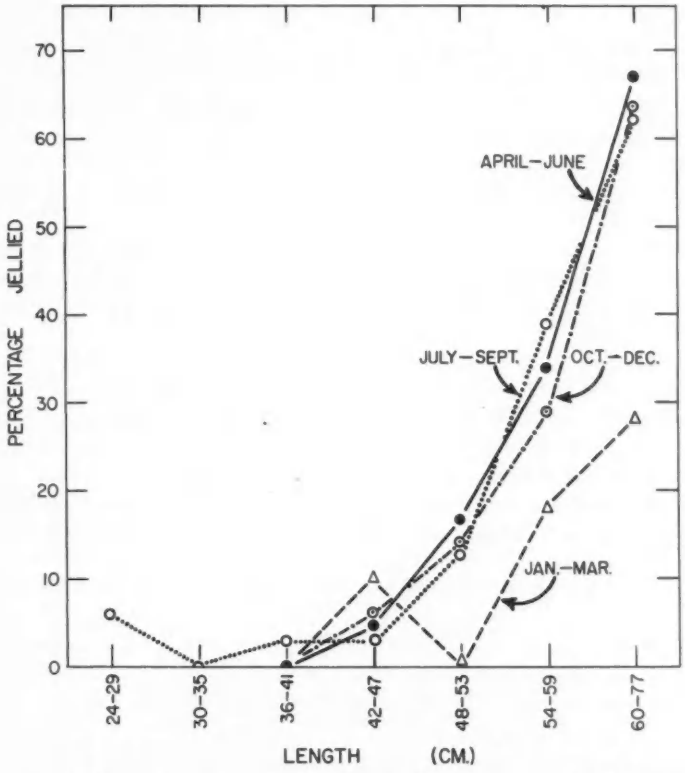

FIG. 4.—Seasonal percentages of jellied plaice from the eastern Grand Bank (areas 8, 9, and 10 of Fig. 2).

Plaice in the area under consideration typically spawn in April–May. Judging by the condition of the ovaries in the winter following spawning and also before spawning the following spring, many of the larger females in this cold area take more than one year between successive spawnings. Thus, in many cases recovery

is slow. However, the seasonal variation does indicate improvement in condition through autumn and winter (Fig. 4).

In the eastern Grand Bank area the trawlers fishing for plaice usually fish in the southern part in winter and move northward during the summer, returning southward again in the latter part of the autumn. Thus, our samples for the different periods of the year are not from the same spot, and hence too strong opinions cannot be held on seasonal differences in a single plaice population. The sampling was, however, indicative of the landings, and shows that the jellied condition can be a very unfavourable factor in commercial exploitation of plaice from April to December. The January to March condition is in doubt, owing to the small numbers examined.

COMPARISON OF JELLIED CONDITION OF PLAICE SAMPLES FROM DIFFERENT DEPTHS AND TEMPERATURES IN THE SAME AREA

On August 29, 1951, during a trip of the *Investigator II* to one of the best plaice fishing grounds on the northeast part of the Grand Bank, samples of plaice in various size ranges were collected from two depths. One lot was taken from 113–116 fath., and the other was from a shallower area, 85–87 fath. deep, only a few miles distant.

Similarly, in the latter part of June, 1953, during an *Investigator II* cruise on the eastern edge of the Grand Bank between 46° and 47°N. latitude, samples of plaice between 51 and 60 cm. were obtained from three depths: 61–63, 80–82 and 122–124 fath.

After landing, in each instance, the plaice were tagged and measured. Those from both depths in 1951 and from all three depths in 1953 were well mixed and afterward filleted and skinned, the fillets being tagged with the same numbers as the fish. The fillets were then examined for condition, the examiner not knowing the depth from which the individual fish yielding these fillets came.

In both experiments, fish from the deeper water gave a considerably higher percentage of normal fillets in every size range (Tables XII and XIII and Fig. 5).

TABLE XII.—Fillet condition of American plaice taken on the northeast Grand Bank, August 29, 1951, at 85–87 and at 113–116 fath.*

Length range	Total fish		Percentage normal		Percentage intermediate		Percentage jellied	
	85–87 fath.	113–116 fath.	85–87 fath.	113–116 fath.	85–87 fath.	113–116 fath.	85–87 fath.	113–116 fath.
cm.								
18–31	0	24	0	100	...	...	...	...
32–43	14	22	100	100	...	...	...	...
44–49	14	13	79	100	21	0	...	...
50–55	15	13	27	100	47	0	27	0
56–61	10	11	0	36	40	27	60	36
62–67	9	10	11	30	22	30	67	40
Total	62	93	48	85	26	6	26	9

*85–87 fath. at 46° 29′ N., 47° 39′ W. and 46° 24′ N., 47° 41′ W.
113–116 fath. at 46° 20′ N., 47° 28′ W.

TABLE XIII.—Fillet condition of American plaice on the northeast Grand Bank, June 1953, at 61-63, 80-82 and 122-124 fath.*

Length range	Number of fish			Percentage normal			Percentage intermediate			Percentage jellied		
	61–63 fath.	80–82 fath.	122–124 fath.	61–63 fath.	80–82 fath.	122–124 fath.	61–63 fath.	80–82 fath.	122–124 fath.	61–63 fath.	80–82 fath.	122–124 fath.
cm.												
51–52	22	19	25	45	68	76	45	26	8	9	5	16
53–54	12	21	17	17	52	94	50	38	0	33	10	6
55–56	22	16	24	27	13	79	55	63	17	18	25	4
57–58	20	22	17	25	18	47	40	45	35	35	36	18
59–60	13	7	13	23	29	38	39	0	23	38	71	38
Total	89	85	96	29	38	70	46	39	16	25	24	15

*122–124 fath. at 46° 23′ N., 47° 35′ W. to 46° 22′ N., 47° 37′ W.
82– 80 fath. at 46° 10′ N., 47° 53′ W. to 46° 07′ 30″ N., 47° 57′ W.
63– 61 fath. at 46° 02′ N., 48° 08′ W.

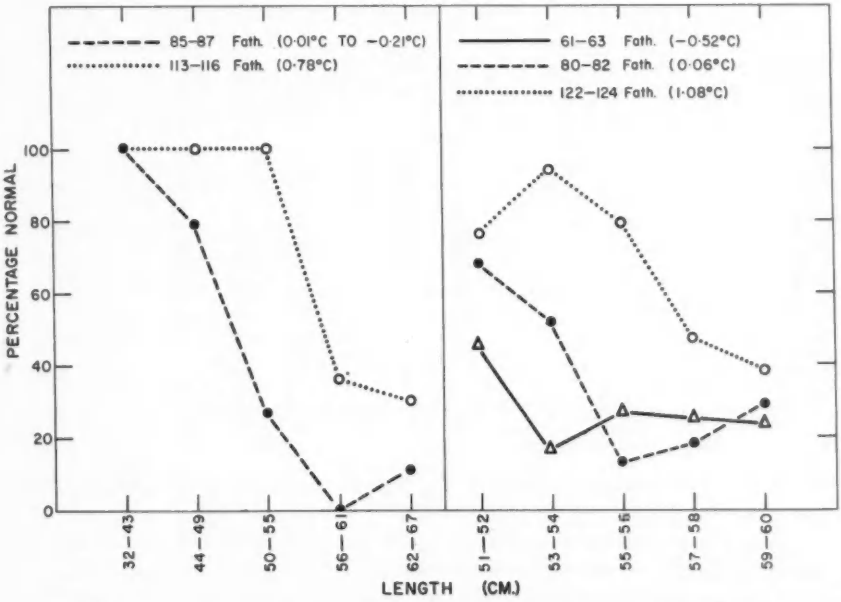

FIG. 5.—Percentages of normal American plaice fillets at different depths and bottom temperatures on the eastern Grand Bank.

The eastern branch of the Labrador Current passes over the eastern slope of the Grand Bank where the large plaice populations are found and where the above samples were obtained. In the otter-trawling sets from which the samples were taken in 1951 the bottom temperatures in the shallower 85- to 87-fath. area were between 0.01 and −0.21°C., while in 113–116 fath. the bottom temperature was 0.78°C. For the 1953 samples, the bottom temperature was −0.52°C. for the 61- to 63-fath. set, 0.06°C. for the 80- to 82-fath. set and 1.08°C. for the 122- to 124-fath. set.

Apparently the slightly higher temperatures in the 113- to 124-fath. water have allowed tissue repair and protein accumulation to produce a higher percentage of plaice with normal tissues at these depths than in shallower colder-water areas.

CONDITION OF PLAICE FILLETS FROM VARIOUS AREAS

The samples of plaice in which fillet condition was examined have been divided into 13 groups. The locations of these samples and groups are shown in Fig. 2, the numbers of fish examined in Table XIV, and the percentages of normal and jellied fillets in Tables XV and XVI. The table of percentage of intermediate type fillet has been omitted as it can be readily obtained from Tables XV and XVI. Table XVII shows the number of plaice 48 cm. and over

TABLE XIV.—Number of plaice examined for condition of fillets from areas 1–13 (Fig. 2 and Tables XV and XVI).

Length range	Number in area													
	1	2	3	4	5	6	8A	7	8	9	10	11	12	13
cm.														
16–19	...	5	44	11	...	...	5	6	2	...	1	29	29	30
20–23	2	18	60	23	...	...	7	17	2	...	3	44	49	55
24–27	6	41	69	12	...	2	6	15	5	...	20	54	44	58
28–31	15	64	58	40	...	3	10	18	9	3	37	73	35	45
32–35	46	76	36	16	...	22	8	34	13	5	39	55	23	30
36–39	89	77	17	4	4	30	8	44	25	10	68	57	28	20
40–43	56	45	5	4	16	29	6	45	49	27	101	63	29	14
44–47	32	29	3	3	8	28	9	23	74	68	280	65	22	14
48–51	17	16	2	1	10	21	24	18	100	58	185	44	32	8
52–55	8	13	...	1	5	20	52	17	140	42	106	44	25	9
56–59	5	5	...	...	...	5	42	12	122	33	78	36	18	4
60–63	...	...	...	...	...	2	11	16	54	22	75	44	7	1
64–67	...	...	...	...	...	...	6	10	21	13	28	26	3	1
68–77	...	...	...	...	...	...	...	2	3	1	24	15	1	...
Total	276	389	294	115	43	162	194	277	619	282	1,045	649	345	289

obtained in the different months of the year for examination of fillet condition. These sizes of 48 cm. and up include most of the jellied fish.

It will be noted that jellied plaice rarely occur at sizes below 44–47 cm. and become a significant and increasing problem in the catches at larger and larger sizes. The percentages of jellied fish are extremely high in the northwestern and the eastern Grand Bank sections (areas 7 to 10). By far the greatest amount of plaice caught in the areas sampled comes from these particular Grand Bank areas. In this region, the highest percentages of jellied fish, for any given size, come from the northwest Grand Bank area. The samples from northwest Grand Bank, area 7, were taken from April to June only (Table XVII) so that it is not possible to draw definite conclusions on relative percentages of jellied between northwest and eastern Grand Bank. The plaice fishery in the northwestern Grand Bank area has been, however, typically a late spring and early summer fishery so that the percentage jellied shown in Table XVI is typical of the actual fishery in the area. It is popularly supposed in the trade that area 8, the northeast Grand Bank area, shows a higher percentage of jellied plaice than the more southern

TABLE XV.—Percentages of normal plaice in different size ranges in various areas of Fig. 2, 1950–53.

Length range	Percentage normal in area													
	1	2	3	4	5	6	8A	7	8	9	10	11	12	13
cm.														
16–19	...	100	100	100	...	...	100	100	100	...	100	100	100	100
20–23	100	100	100	100	...	...	100	100	100	...	100	100	100	100
24–27	100	100	100	100	...	100	100	100	100	...	100	98	98	100
28–31	93	100	100	100	...	100	100	100	100	100	97	100	100	100
32–35	98	100	100	100	...	100	100	100	100	100	100	100	96	100
36–39	98	97	100	100	100	100	100	95	100	100	99	100	100	95
40–43	88	100	100	100	100	100	100	87	96	96	96	95	90	93
44–47	81	100	100	100	100	100	100	70	78	82	69	91	86	71
48–51	76	75	100	100	90	100	83	33	63	55	55	93	75	63
52–55	50	77	...	100	40	85	87	0	33	26	36	75	60	44
56–59	20	80	...	...	...	20	52	8	18	27	22	53	22	25
60–63	...	...	...	...	...	100	36	6	13	5	17	27	14	0
64–67	...	...	...	...	...	...	17	0	0	8	18	15	0	0
68–77	...	...	...	...	...	...	...	50	0	0	4	7	0	...
Of total fish	90	97	100	100	91	96	78	71	48	55	60	83	86	93
Total fish	276	389	294	115	43	162	194	277	619	282	1,045	649	345	289
Depth, fathoms—														
Shallowest	94	104	80	38	120	114	113	57	63	25	30	34	25	34
Deepest	140	230	196		148	183	124	90	104	85	85	115	120	110
Temperature, ° C.—														
Lowest	−0.29	0.29	−1.15	0.04	3.14	−0.98	0.78	...	...	...	...	...	−0.61	0.13
Highest	2.14	2.45	1.51		3.42	2.09	1.08	...	...	...	...	...	3.66	3.71

TABLE XVI.—Percentages of jellied plaice in different size ranges in various areas of Fig. 2, 1950–53.*

Length range	Percentage jellied in area													
	1	2	3	4	5	6	8A	7	8	9	10	11	12	13
cm.														
16–19	...	0	0	0	...	...	0	0	0	...	0	0	0	0
20–23	0	0	0	0	...	...	0	0	0	...	0	0	0	0
24–27	0	0	0	0	...	0	0	0	0	...	0	2	0	0
28–31	0	0	0	0	...	0	0	0	0	0	3	0	0	0
32–35	0	0	0	0	...	0	0	0	0	0	0	0	0	0
36–39	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40–43	0	0	0	0	0	0	0	0	0	4	0	0	0	0
44–47	0	0	0	0	0	0	0	13	5	3	7	5	0	0
48–51	0	0	0	0	0	0	13	17	11	5	13	2	0	13
52–55	12	8	...	0	0	0	6	59	20	29	27	9	8	22
56–59	20	20	...	...	...	0	17	67	35	49	36	17	17	75
60–63	...	...	...	...	...	0	45	81	59	59	52	27	71	100
64–67	...	...	...	...	...	...	67	100	86	69	36	35	100	100
68–77	...	...	...	...	...	...	...	50	100	100	79	67	...	...
Of total fish	0.7	0.5	0	0	0	0	11	17	22	20	16	7	4	3

*Values for Total fish, Shallowest and Deepest Depths, and Lowest and Highest Temperatures, same as in Table XV.

TABLE XVII.—Number of plaice 48 cm. and over in Tables XV and XVI caught in various months.

Month	Number in area													
	1	2	3	4	5	6	8A	7	8	9	10	11	12	13
January	...	...	...	...	...	...	...	...	...	...	53	55	...	...
February	...	...	...	...	...	...	...	...	...	...	...	...	...	...
March	...	...	...	...	...	...	...	...	...	...	...	39	57	...
April	...	...	...	...	...	...	...	10	...	29	36	75	...	...
May	...	...	...	...	...	...	...	45	...	...	27	23	...	21
June	...	...	...	...	...	...	97	20	302	...	20	...	19	...
July	...	...	...	...	15	26	...	...	39	...	32	...	7	...
August	...	...	...	...	...	...	38	...	71	88	25	...	...	...
September	30	34	...	...	...	...	...	...	28	18	49	...	...	...
October	...	...	...	...	...	...	...	...	...	...	123	...	...	...
November	...	...	2	2	...	23	...	...	...	34	64	17	...	2
December	...	...	...	...	...	...	...	...	...	...	67	...	4	...
Total over 48 cm.	30	34	2	2	15	49	135	75	440	169	496	209	87	23

Grand Bank areas (9 and 10). Tables XV and XVI, however, do not show any very significant differences between these areas except at the very largest size ranges where the numbers sampled are small. At these sizes, above 64 cm., there is an increasing percentage of jellied from south to north along the eastern edge of the Grand Bank.

In the St. Pierre Bank–Fortune Bay area (12) and the Banquereau–Cape Breton area (13) the percentages of plaice jellied were very high at the largest sizes; but very few of these large plaice were present in the Banquereau–Cape Breton area, and from the St. Pierre Bank–Fortune Bay area few fish above 60 cm. were examined.

The deep-water area (8A) (113–124 fath., 0.78 to 1.08°C. temperature) to the northeast of the Grand Bank has considerably lower percentages of the plaice jellied than have shallow water (63–104 fath.) samples from the same general area (8). Samples from the southwestern Grand Bank area (11) (30–115 fath.), where in general there are warmer water conditions than on the eastern edge of the Grand Bank, have considerably higher percentages of normal fish and lower percentages of jellied ones at the same sizes than the fish from the eastern and northwestern areas of the Bank (7, 8, 9, 10). Over the deeper, warmer parts of the southwestern Grand Bank, however, plaice are scarce, particularly the larger sizes.

In the areas of Hamilton Inlet Bank (1), east of Belle Isle Bank (2), Notre Dame Bay (3), Bradelle Bank (4) and Flemish Cap (5) most of the fish were small or medium sized and very few were jellied. The small number of large fish present had a considerably lower percentage of jellied than in the eastern and northwestern areas of the Grand Bank, although among the large fish from Hamilton Inlet Bank there was a high percentage in the intermediate stage. In the deep-water sample off Cape Bonavista (6) there were moderate numbers of fish 52 cm. and over but none were jellied.

DISCUSSION AND CONCLUSIONS

Many of the Grand Bank samples in Tables XIV, XV and XVI were from commercial vessels and, in these cases, bottom temperatures are not available. The remainder are from the exploratory cruises of the *Investigator II* and bottom

temperatures were usually taken. From hydrographic cruises temperature conditions generally are well known for the Grand Bank area.

On the northwest and east Grand Bank the greatest stocks of the larger plaice live on the slope area in about 60 to 90 fathoms, in the path of the western and eastern branches of the cold water of the Labrador current, and temperatures over a large part of the year are below 0°C. and often as low as —1°C. These conditions are apparently favourable for the general life history of the species, since plaice are very numerous. In fact, in the area under discussion plaice is the dominant large fish in the water below 0°C. Sexual maturity is at a large size and very large, very old fish are abundant. Here also, however, with the abundance of large fish, the jellied condition is common. There are higher temperature areas adjacent to the colder northwest and east Grand Bank areas. These areas are the deeper water of the northeast Grand Bank (8A), 113–124 fath., with bottom temperatures 0.78°C. to 1.08°C.; off Cape Bonavista 114–183 fath., bottom temperatures 0.98° to 2.09°C.; Flemish Cap (5), 120–148 fath., bottom temperatures 3.14° to 3.42°C.; and the southwestern edge of the Grand Bank (11) which, in depths below 50 fath. and usually below 30 or 40 fath., has higher temperatures all year round than the eastern and northwestern slopes of the bank. Area 2, east of Belle Isle Bank, 104–230 fath. and temperatures 0.29°C. to 2.45°C., is a similar area. In these warmer areas, there are usually fewer large plaice, fewer jellied individuals among the larger fish, but also usually far fewer plaice than in the colder water areas.

In the remaining areas the situation is not so clear, both from lack of large fish in the samples in areas 1, 2, 3, 4, and 13, and the lack of adequate samples of large fish from different depths and temperatures in the same general area.

PLAICE WEIGHBACKS, ST. JOHN'S, NEWFOUNDLAND

Weighbacks, 1952

Table XVIII shows catches and weighbacks (fish discarded at plant and deducted from the vessel's landings) from plaice catches by St. John's trawlers

Table XVIII.—Weighback for jellied condition, from commercial plaice landings, St. John's, 1952.

Depth	Number of trips			Average gross landings in 1,000's pounds*			Average percentage weighback*		
	NW. side	SW. portion	Eastern side	NW. side	SW. portion	Eastern side	NW. side	SW. portion	Eastern side
fath.									
31–35	...	...	2	...	...	128	...	...	10
36–40	...	...	12	...	...	128	...	...	8
41–45	1	2	13	(7)	5	88	(18)	14	7
46–50	2	...	3	14	...	119	16	...	7
51–55	2	...	2	104	...	98	22	...	6
56–61	6	...	2	158	...	136	29	...	24
66–70	4	2	2	118	16	137	21	3	39
71–75	10	...	1	124	...	(106)	20	...	(15)
76–80	7	...	2	88	...	152	19	...	42
81–85	3	...	...	66	...	...	21	...	...
86–90	1	...	...	(73)	...	...	(12)	...	...
91–95	...	2	...	...	35	...	...	4	...
96–100	...	...	...	...	...	...	...	...	...

*Values in parentheses indicate data from only one catch.

on the Grand Bank in 1952. The weighback includes an allowance for flounders discarded for any reasons whatever: for example, small size, softness, bruised fillets, as well as the jellied condition. Generally, however, especially when the plaice weighback is large, it is mainly due to the discarding of jellied plaice. Very few large plaice were discarded at sea in 1952, and hence the percentage weighbacks of plaice are a fairly good index of the relative quantities jellied on the various fishing grounds sampled.

On the northwest part of the Grand Bank, which is a low-temperature area covered by the western branch of the Labrador Current, the weighback does not appear to be very significantly different in catches of plaice from 41 to 85 fath., averaging 20% at all these depths apart from 56 to 61 fath. where the average weighback was 29%. The plaice fishery in this northwestern area was almost entirely in the April to June period. The greatest catches of plaice were between 51 and 75 fath.

The southwestern portion of the bank, which has moderately high temperatures throughout the year, is not typically a plaice area and only small incidental catches are obtained there. In fact, if haddock fishing is good, the few plaice caught in this area are often discarded. The deeper the water in this area, the higher the temperature. In consequence, agreeing with the general evidence that the jellied condition in plaice is due to emaciation and lack of recovery from spawning at very low temperatures, on the southwestern part of the bank there is no great weighback at any depth due to jellied condition.

On the eastern slope of the Grand Bank most of the plaice fishing in 1952 was in the July to September period. This is a great commercial fishing area for plaice and is a cold-water area covered at intermediate depths by the eastern branch of the Labrador Current. The weighback was very small from the shallower depths of 31 to 55 fath. and much greater weighbacks, probably increasing generally with depth, occurred in catches from 56 to 80 fath. On this eastern edge, there were good catches of plaice at all depths fished. The larger weighbacks coincided with an increased size of the plaice in the deeper water. On the basis of research ship samples, we would expect less jellied plaice from catches in still deeper and warmer water below 100 fath. on the eastern edge of the bank. Plaice however, are not usually so plentiful in water of 100 fath. and deeper, as at shallower depths.

Weighbacks, 1953

In 1953 information was gathered with more detail than in 1952, on the amounts of American plaice weighed back from trawler landings at St. John's and sent to the meal plant. All catches used in Table XIX are from the southern part of the eastern edge of the Grand Bank and are in all cases weighbacks mainly for jellied condition, as determined by statements from the plant.

In the catches from 43 to 101 fath. the smallest average sizes are at the shallower depths, approximately between 40 and 60 fath. At depths beyond 70 and up to 100 fath. larger fish are caught, and with rare exceptions there is a considerably greater weighback than at the shallower depths. However, due

TABLE XIX.—Weighback for jellied condition, from commercial plaice landings, St. John's, 1953, from the southeastern part of the Grand Bank.*

Depth	Date 1953	Gross landings	Per cent weighback	Average landed length	Per cent number landed 50 cm. & over	Per cent number landed over 55 cm.	Per cent weight landed over 55 cm.	Remarks
fathoms		*1000's pounds*		*cm.*				
43 (35–55)	Sept. 2–8	125	2.4	43.8	9.0	2.5	7.2	. . .
48 (47–60)	Sept. 24–28	176	2.6	45.7	15.5	2.0	5.2	No large discarded at sea
50 (47–60)	Oct. 4–10	175	3.1	44.0	17.5	4.0	8.7	Only a few large discarded at sea
50 (45–70)	Nov.4–11	185	2.0	44.5	9.1	1.9	4.6	No large discarded at sea
50 (44–60)	Sept. 12–20	196	4.7	45.5	21.0	6.8	13.7	No large discarded at sea
70 (55–85)	Nov. 15–18	187	2.8	49.1	38.0	13.0	23.0	No large discarded at sea
73 (65–85)	Nov. 9–15	188	30.8	53.8	81.3	38.5	49.9	Only extra large discarded at sea
75 (65–90)	Nov. 21–30	129	2.6	50.1	44.4	19.8	31.6	No large discarded at sea
78 (60–85)	Aug. 22–25	140	14.0	49.8	52.0	13.0	20.3	No large discarded at sea
80 (65–91)	Sept. 27–Oct. 3	133	28.5	49.1	48.3	17.9	31.0	No record of discard at sea
81 (70–92)	Sept. 30–Oct. 5	147	16.1	49.5	45.2	16.7	27.1	9,000 to 10,000 lb. large discarded at sea
82 (68–98)	Aug. 6–12	170	12.4	49.5	49.8	14.9	24.2	15,000 lb. large discarded at sea
101 (83–120)	Aug. 9–14	165	33.2	50.2	57.6	18.7	28.7	No record of discard at sea

*The southeastern area of the Grand Bank used here is bounded in the north by latitude 46° N., in the south by latitude 42° 45′ N. and in the west by longitude 50° 30′ W. Each line represents data for one trip of one ship.

allowance must be made for the very large fish which were discarded at sea. Considering all depths, it is evident that there is usually a close relation between the weight of plaice over 55 cm. landed and the percentage weighback. There are some exceptions, however, and more observations are obviously needed to explain exceptional cases. The shore measurements on which the percentages landed over 50 and over 55 cm. were calculated are relatively small compared to the total numbers of fish landed from a trip and there are thus possibilities of failure to assess accurately the sizes of fish in the individual catch. Many factors also such as the length of the trip, the quality of the icing and the need of the plant for fish may affect the percentage discarded on shore.

PARASITES AND JELLIED CONDITION

MILKY CONDITION OF VARIOUS FISHES

A milky condition has long been known to occur in the South African stockfish *Merluccius capensis*, the snoek *Thyrsites atun*, the John Dory *Zeus faber*, and in the Australian barracouta *Thyrsites atun*. Johnston and Cleland (1910), Gilchrist (1924), Davies and Beyers (1947) and Willis (1949), each working with one or more of these species, have shown that when milkiness is present, a myxosporidian parasite *Chloromyxum thyrsites* is also always present in the muscle. In the diseased specimens, the flesh softens in a few to 24 hours after capture and finally disintegrates into a thick viscous mass (Willis, 1949). More recently Fletcher, Hodgkiss and Shewan (1951) have described a similar milky condition, associated with the presence of *Chloromyxum* sp., in hake landed in Great Britain from the fishing grounds off the coast of northwestern Africa.

Thompson (1916) described from landed catches of Pacific halibut specimens with soft and mushy flesh which in its advanced stages was milky. He mentions his inability and that of the fishermen to distinguish mushy or milky fish when freshly caught. He describes a sporozoan, in shape like a four-pointed star, with four polar capsules, which was found associated with the mushy condition.

M'Gonigle and Leim (1937) found *Chloromyxum* sp. associated with a "jellied" condition in a swordfish, *Xiphias gladius*, from the Canadian Atlantic coast. Arai and Matsumoto (1953) found the sporozoan *Hexacapsula neothunni* associated with "jellied" muscle of yellowfin tuna landed in Japan. Margolis (MS., 1953) mentions that *Chloromyxum* sp. is apparently the agent which causes a softening and liquefaction of the muscles of the lemon sole, *Parophrys vetulus*, in British Columbia. This condition is present among the British Columbia lemon sole populations, with heavier infections in some areas than in others. Matsumoto (1954) described two new sporozoa, *Chloromyxum musculoliquefaciens* and *Neochloromyxum cruciformum*, which he found responsible for a jellied condition in the muscle of the swordfish, *Xiphias gladius*, and of the common Japanese sea-bass, *Lateolabrax japonicus*.

In the milky or so-called "jellied" fish described above the flesh was white and milky in appearance. Some of the muscle was pulpy and disintegrated. The flesh could not be filleted and could be squeezed out through a cut surface like toothpaste.

Typically, the fish which are milky on landing are firm when caught, and it is likely (Willis, 1949) that *Chloromyxum thyrsites* secretes a powerful proteolytic enzyme which in life is removed by the blood stream but which after death can cause disintegration of the protein.

Jellied American Plaice

Jellied plaice muscle does not have the appearance described above. It is flabby but even in the worst condition is neither disintegrated nor milky nor similar to toothpaste in consistency. It can be filleted readily. It is whiter and more glossy than normal plaice flesh but not milky white in appearance. The essential features of the jellied condition, a large amount of water (or of water plus fat) and a small amount of protein, are present in the fish when caught. The flesh of badly jellied fish can be recognized as jellied immediately after capture although not so readily as later. Doubtless, jellied plaice which have been iced for a long time are flabbier in appearance than newly caught fish; but the water content of such iced fish when landed is lower, not higher, than when the fish were captured. In the jellied plaice there is no evidence of the milky areas embedded in the flesh which are so characteristic of the milky fish infected with *Chloromyxum*.

In a number of longitudinal and transverse histological sections of the muscle of 12 normal and 13 jellied plaice, in spite of very detailed examination of many sections under oil immersion, no sporozoan parasites were found. These sections were also examined by Dr. L. Margolis, parasitologist at this Board's Biological Station at Nanaimo, British Columbia. Dr. Margolis confirmed our observations. He reported that no spores of *Chloromyxum* were present and he was unable to find evidence of *Chloromyxum* infection.

Fluorescence Under Ultra-violet Light

Davies and Beyers (1947) show that muscle fibres infected with *Chloromyxum* fluoresce under ultra-violet light filtered through Woods glass and that

even deeply embedded infections can be readily detected. Even a single infected fibre in a whole fish can be detected by fluorescence. The extent of the infection may vary from a single muscle fibre to a state in which the whole flesh is permeated by fluorescing fibres.

Examination is conducted on the fillet or sometimes on the flat cut surface of the fish cut in two halves and with the vertebral column removed.

On October 23, 1953, seven normal and two extremely jellied plaice fillets, and on November 13, six normal and 12 definitely jellied plaice fillets, all from the commercial grounds on the eastern edge of the Grand Bank, were examined in a dark room under an "Alpine" ultra-violet lamp of 140 watts with a Woods glass filter to cut out visible but transmit ultra-violet light. There was no evidence whatever of fluorescence due to sporozoan infection. Some small cysts fluoresced brilliantly in both the normal and the jellied flounder, but these were all found to be trematode larvae.

HISTOLOGY OF NORMAL AND OF JELLIED PLAICE FILLETS

In sections of 12 normal and 13 jellied plaice obtained (with both jellied and normal representation in each case) in December 1949, July 1952, August 1952, and September 1953, the following fairly consistent differences were found:

(a) Greater spaces are apparent between the muscle fibres of jellied plaice (Fig. 6) than of normal plaice (Fig. 7). Thus, there is less muscle fibre material per unit of area in the sections of jellied fillets than in the sections of normal fillets.

(b) In longitudinal section, the muscle fibres of the jellied plaice generally exhibit a waviness —the result of coiling—not apparent, beyond an occasional slight waviness, in the sections of the muscle of normal plaice. This coiling possibly represents the effect of fixation on the looser structure of the jellied tissue.

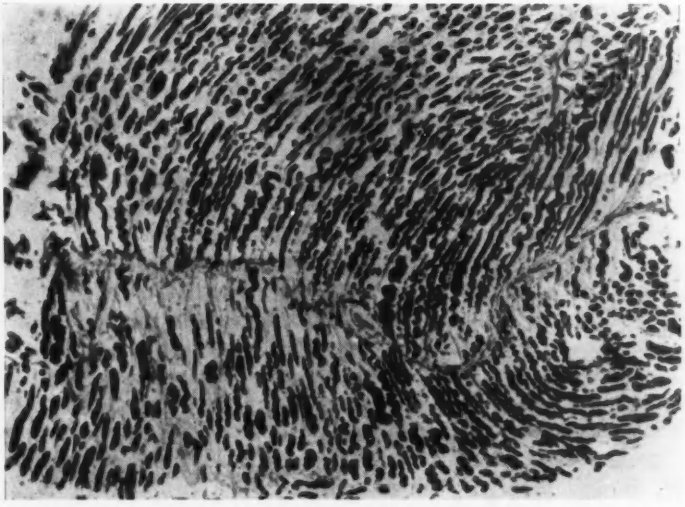

FIG. 6.—Longitudinal section of portion of fillet of jellied American plaice.

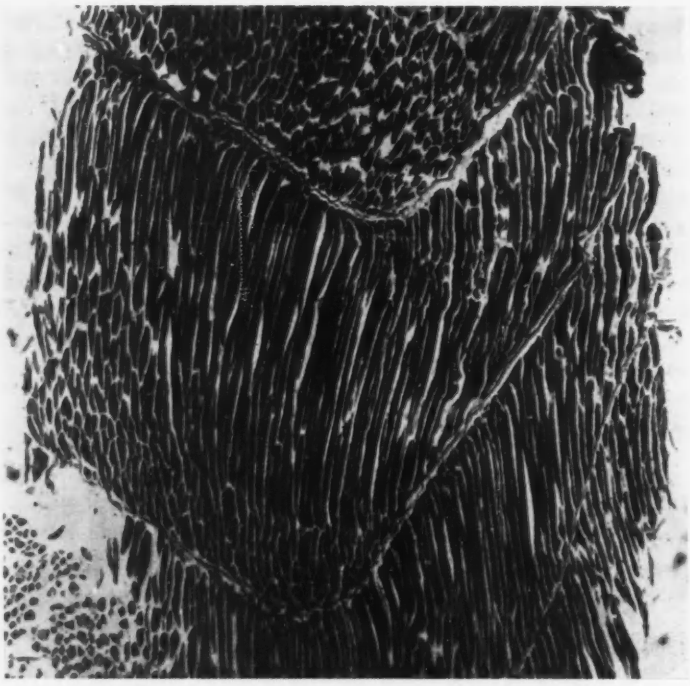

FIG. 7.—Longitudinal section of portion of fillet of normal American plaice.

COOKING AND TASTE TESTS ON PLAICE FILLETS

Several experiments were performed on the cooking and tasting of normal, intermediate and jellied plaice fillets. Fillets were at first cooked in deep fat in cheesecloth bags but there was difficulty in separating the cooked fillet from the cloth for weighing. More satisfactory results came from cooking pieces of the same size in deep fat, heated to approximately the same temperature.

Each piece of fillet, approximately 100 grams, was cooked until it was indicated by the external browning that cooking was complete. This required 36 minutes for 112 grams of normal plaice and 45 minutes for 107 grams of jellied plaice. The weight loss was 37.5% in the case of the normal and 45.8% for the jellied plaice. In the normal fillet, the flavour was excellent and typical of flounder. The flesh was well cooked and rather dry. In the jellied fillet, the centre was still jelly-like and rather unacceptable as food because of its gelatinous texture, although the taste was not objectionable. The external brown part was of excellent flavour. The jellied fillet would thus require much longer cooking, would shrink considerably and might have a jelly-like centre even after prolonged cooking. Smaller pieces of the jellied flounder, cooked brown in deep fat in the same way as scallops or cod tongues, were of excellent texture and flavour although there was considerable shrinkage.

The intermediate type fillet was of good texture and was not quite as dry as the normal after cooking. In several cooking tests, the product produced from the intermediate variety of fillet was very acceptable as food. The ordinary commercial pack, judging from the moisture frequencies found in the "drip experiment", includes both the normal and intermediate categories.

JELLIED CONDITION IN FISH OTHER THAN AMERICAN PLAICE

While we have no analytical data on jellied condition in other fish, a similar but usually less extreme condition probably occurs.

Dr. W. F. Royce, formerly chief of the North Atlantic Fishery Investigations, United States Fish and Wildlife Service, says that investigators who have sampled a large quantity of yellowtail and blackback flounder in the New England area have neither seen nor heard of a jellied condition in these species (personal communication, 1950).

Mr. Joseph F. Puncochar, bacteriologist in charge of the United States Fish and Wildlife Service's technological laboratory in Boston, says that after checking with several firms he finds that the jellied condition is not encountered in American plaice from the New England area (personal communication, 1950). He has heard of a condition, however, in yellowtail and blackback flounder fillets that is similar to the jellied condition.

Mr. Homer Zwicker of Zwicker & Company, Lunenburg, Nova Scotia, states that in addition to plaice he has seen haddock in the same jellied condition (personal communication, 1953).

Mr. H. P. Connor of the Maritime-National Fish Company, Halifax, writes that the jellied condition has not been noted in yellowtails or grey-sole at the Halifax plant (personal letters, 1953).

We have seen a type of jellied condition, but not so extreme as that in the plaice, in very large old cod from deep cold water, about 70 fath., off St. John's. Dr. H. Fougère of this Board's Technological Station at Grand River, Que., reports the occurrence of similar large jellied cod from deep water off Gaspé.

Bogucki and Trzesinski (1950) indicate that in cod from the Gulf of Gdansk the water content of the muscles is highest, often over 83%, in the period from May to September, while it is lowest, 80.4 to 81.5%, from October to April. This is suggestive, but since between these seasons there may have been variation in size of fish sampled, the data given are insufficient for proof of a jellied condition.

Bailey (1950, 1951) has shown interesting relations between percentages of protein, oil and water in the flesh of normal, "chalky" and very chalky specimens of the Pacific halibut. With increasing chalkiness the fish has a lower percentage of water and higher percentages of protein and of oil. This chalky condition of the flesh is, in relation to water and protein content, apparently the opposite of the jellied condition in the American plaice.

SUMMARY AND CONCLUSIONS

1. American plaice are, as a rule, largest and most numerous in cold water, approximately $-1°$ to $+1°$C. In the Newfoundland area they are thus very

plentiful on the northwestern, northern and eastern parts of the Grand Bank in the path of the Labrador Current. In the Maritime area they are abundant in the northern colder water parts of the southern Gulf of St. Lawrence. The Canadian catch of plaice in the Newfoundland area (Subarea 3 of ICNAF), from a small beginning in 1948, is now about 30 million pounds a year. In the Nova Scotian–Gulf of St. Lawrence area (Subarea 4 of ICNAF), there is an annual catch of approximately 15 million pounds, of which about 12 million pounds come from the Gulf of St. Lawrence.

2. In the new fishery for plaice in the Newfoundland area there are considerable losses due to a jellied condition. Sometimes as much as 20 to 40% of the catch may be discarded on landing as jellied. Since the jellied condition cannot be detected in whole fish, the condition is usually found only when the fillets have been removed. Thus, there is a raw material loss and a loss of shore labour also. Customers object to the jellied fish because of its appearance, jellied condition when cooked, and shrinkage during cooking.

3. For descriptive purposes, ordinary fillets, without question commercially acceptable, were designated as "normal". These were firm and with a greyish colour in the high quality smaller fillets but with a whiter colour in fillets from larger fish. The jellied fillets were those which without question should have been rejected in a plant. They were jelly-like, glossy and opalescent. An "intermediate" condition was also recorded in which fillets could generally be used commercially but with some doubt.

4. Jellied fillets of American plaice have on the average over 4% more water and over 4% less protein than normal fillets. Fat is only slightly (0.2%) higher in the jellied than in the normal fillets (Table V). In terms of actual percentages of the normal, the moisture increase is 5%, the fat increase 38%, and the protein decrease 29% in jellied fillets. The moisture and protein average differences between jellied and normal are very highly significant and the fat difference also is statistically significant.

If the fat content is high, the jellied condition can be present in a fish of a very low moisture content. There is, therefore, less overlapping between jellied and normal, if moisture and fat are considered as one factor. Although the average fat may be 0.2% higher in the jellied fillets, plaice of abnormally high and also abnormally low fat content, up to almost ten times the average and down to one-quarter of the average fat content, may be either normal or jellied.

The protein content, however, allowing for a small overlap due to selection by sight and touch, is consistently high in normal and low in jellied fillets. From the analytical point of view of chemical composition, therefore, the significant difference between jellied and normal fillets is the relatively low protein content of the jellied fillets.

5. Protein fractionation shows the muscle myosin fraction, which is most important in the muscle fibre itself, to be considerably reduced in the jellied flounder. The stroma, which includes the connective tissue, shows an increase in the jellied flounder, when stroma nitrogen is considered as a percentage of total protein nitrogen. It is indicated that, while both muscle fibre material and connective material have decreased in total quantity in the jellied fillets, the

connective tissue has not decreased by nearly as great a fraction as the muscle fibre material. There is no evidence of proteolysis or of the accumulation of proteolytic end-products such as would be expected if the sporozoan parasite *Chloromyxum* were the causal agent of the jellied condition.

6. Normal and jellied half-fillets, collected in sealed bottles at sea, and kept unfrozen on ice, show an average liquid drip in the bottles on landing of 14.4% (by weight) from the jellied, and 2.1% from the normal fillets. The remaining half fillet in each case was left on the fish in ice. On shore, compared with the total moisture content of the sea samples, the jellied fillets in ice from 2 to 6 days lost almost 1% moisture, while the normal fillets in ice increased 0.7% in moisture content. One of the fillets from this experiment had the extremely jellied composition of 96.18% water, 0.06% fat and only 2.83% protein when collected at sea.

7. The analyses indicate that the commercial pack includes both normal and intermediate varieties. Quick-frozen jellied plaice fillets have considerably more drip on thawing than the commercial pack. This drip is considerably greater than could be accounted for by the difference in moisture content of the fillets. The probable explanation is that in the jellied fillets, which have a looser, less firm physical structure, more of the moisture is in the form of lymph in enlarged inter-cellular spaces and consequently this fluid more readily runs out of the tissues.

8. Examination of 2,406 plaice in sizes from 16 to 77 cm. and from the northwestern, northeastern and eastern sections of the Grand Bank has shown that the jellied condition is negligible in the immature fish. It occurs with increasing frequency in the larger male and female sizes. Since males do not grow to as large a size as females, by far the greater weight of jellied fish in a commercial catch is due to the presence of large female fish. Most of the females above 60 cm. in length are jellied and very few are normal.

9. The average weights of normal and jellied plaice of the same length are only slightly different. Thus, weight differences cannot be used to separate jellied from normal plaice before filleting.

10. On the eastern part of the Grand Bank, large fish show only slight recovery in flesh condition from April to December. There is a possibility of some recovery from January to March, but the observation is of doubtful value from the smallness of the sample and the more southerly location. Spawning is typically in April–May.

11. On the eastern edge of the Grand Bank, there is a considerably higher percentage of normal and a correspondingly lower percentage of jellied fish in the same size ranges in deeper and warmer water (113–124 fath., 0.78 to 1.08°C.) than in shallower and colder water (61–87 fath., —0.52 to 0.06°C.).

12. By an examination of plaice samples from many areas of the northwest Atlantic, it was concluded that where the bottom temperatures are low, from —1° to 0°C., during most of the year (for example on the northwestern, northern and eastern edges of the Grand Bank) the plaice are large and often plentiful and have a high percentage of jellied fish at the larger sizes over most or all of the year.

In deeper or warmer areas such as the southwest Grand Bank, the deeper

water to the north of the Grand Bank, and Flemish Cap, temperatures are higher, and large and jellied plaice are considerably less plentiful than in the colder areas. Often these favourable temperatures are only 1 to 3°C.

13. Weighbacks on landing, chiefly due to jellied condition, in plaice from the northwestern part of the Grand Bank in April–June, 1952, were typically about 20%. The percentage jellied did not show significant differences with depth, between 41 and 85 fath.

On the eastern edge of the Grand Bank in 1952 and 1953, weighbacks for discarding due to jellied condition were at a low level in catches from a depth of 31 to 55 fath. but showed increases at greater depths down to 100 fath. Plaice sizes were smaller in shallower water, and larger in the deeper water, and the amounts discarded on landing were usually closely related to the weight of plaice over 55 cm. in length landed.

14. From many fishes there is evidence of a "milky" or so-called "jellied" condition due to myxosporidian parasites, usually *Chloromyxum*. From the physical appearance and the chemical composition of the fillets, lack of fluorescence in the fillets under ultra-violet light, and lack of parasites in sections of jellied fillets, no evidence was found to indicate that the jellied appearance of American plaice was due to infection by a sporozoan or other parasite.

15. Histological sections showed less muscle fibre material and greater interfibre spaces in jellied than in normal fillets. A much greater waviness, possibly due to shrinkage on fixation, is seen in the muscle fibre in longitudinal sections of jellied than of normal plaice.

16. Cooking tests in deep fat showed that the jellied fillet underwent considerably more shrinkage than the normal and might have a jellied centre after prolonged cooking. Owing to the greater water content, cooking took longer for the jellied fillet. Small pieces of jellied fillet cooked in deep fat were of excellent texture and flavour.

17. Through private communications and from personal observations there is some evidence that cod and haddock and possibly some flatfish species other than plaice sometimes are subject to a jellied condition. The condition in these fishes is, from the information available, likely to follow the spawning season and in the cod to be often prolonged for a considerable time after spawning, if the fish is living in deep and extremely cold water. In none of these fishes is the problem more than an incidental occurrence; and of the commercial groundfishes in the Western Atlantic, only in the plaice is there a very definite and extreme jellied condition in a large part of the population and throughout the year.

18. As a result of our work on many phases of the problem of jellied plaice, the hypothesis is advanced that the jellied condition is one of protein emaciation or impoverishment. The cause is related to spawning; namely, failure of fish living in very cold water to recover the body protein loss to the sexual products before the gonads with first call on the blood protein are developing again. There is a resulting need for more protein of which the first source is the food and secondarily the body protein. As the protein declines, water is absorbed to replace it. The actual physical appearance of the flabby jellied condition is

attributed to the decrease in quantity of muscle fibre material. Also, there is an increase in inter-fibre spaces, with a greater total quantity of lymph in these spaces than in the more solid normal tissue.

This hypothesis is supported by the following arguments:

(a) The jellied condition is prevalent only in the larger of the sexually mature fish.

(b) The jellied fillets have considerably less protein than the normal fillets and there is a gradual decline in percentage protein from the smaller to the larger plaice.

(c) Jellied fish are common throughout the year in areas where the fish are very old and large and where the bottom water for a great part of the year is between −1° and 0°C. They are less common, year round, where average temperatures are as high as 1°C., and with higher temperatures still, only few jellied fish occur. Apparently, the rate of digestion and tissue repair is a factor in the occurrence of the jellied condition.

(d) The myosin fraction or muscle fibre fraction is reduced in jellied fillets, while the stroma containing the connective tissue—though somewhat decreased—is not at all decreased in as great a proportion as the myosin fraction. In microscopic section of fillet tissue, muscle fibre material occupies less area, and the spaces between the fibres more area, than in normal tissue.

(e) There is no indication from gross or from microscopic structure that the jellied condition is produced by sporozoan or other parasites. There is no evidence of protein disintegration. The high water and low protein contents of jellied fillets have been shown to be characteristic of the fish when caught.

(f) Weights of otherwise comparable normal and of jellied fish are approximately the same. The loss of protein is compensated for by increase in water or in some cases fat.

19. The jellied condition is so prevalent (over 50%) in plaice of 60 cm. and over from the eastern and northern Grand Bank that it is probably cheaper to discard these large sizes at sea, but only as dead fish. Labour costs and the policy of the plant with regard to using the intermediate types will determine whether it is worth while to bring in and fillet the 56- to 59-cm. or the 52- to 55-cm. fish. If most would be thrown out in the plant after the added cost of icing, shore handling and filleting, they should be discarded at sea. These plaice are very old; often, as shown by Mr. R. S. Keir's studies of plaice ages at this Newfoundland Station, 20 to 40 years of age.

20. The Grand Bank area, where the large and jellied plaice are numerous, is a new fishing area for American plaice and intensive fishing for a few years should reduce the percentages of jellied fish and allow the fish of smaller and intermediate sizes to increase in number. The smaller fish between 30 and 38 cm. possess grey fillets of excellent quality with high protein and low water content. Utilization of these smaller fish is indicated as far as the economics of filleting allow and will in any case be necessary as the very large fish are reduced in numbers.

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REFERENCES

Arai, Y., and K. Matsumoto. 1953. On a new sporozoan, *Hexacapsula neothunni* gen. et sp. nov., from the muscle of the yellowfin tuna, *Neothunnus macropterus*. *Bull. Japanese Soc. of Scientific Fish.*, **18**(7): 293–298.

Bailey, B. E. 1950. "Chalky" halibut. *Fish. Res. Bd. Canada, Pac. Prog. Rep.*, No. 84, pp. 68–69.

1951. "Chalky" halibut. II. *Ibid.*, No. 88, p. 61.

Bogucki, M., and P. Trzesinski. 1950. Fluctuations in the water and fat content of the cod. *J. du Cons.*, **16**(2): 208–210.

Cutting, C. L. 1951. Loss of weight and shrinkage of iced fish on trawlers. *Fishing News*, No. 1975: 10–13.

Davies, R., and E. Beyers. 1947. A protozoal disease of South African trawled fish and its routine detection by fluorescence. *Nature*, **159**: 714.

Dyer, W. J., H. V. French and J. M. Snow. 1950. Proteins in fish muscle I. Extraction of protein fractions in fresh fish. *J. Fish. Res. Bd. Canada*, **7**(10): 585–593.

Fletcher, L. I., W. Hodgkiss and J. M. Shewan. 1951. The milkiness of Mauretanean hake and its probable cause. *The Fishing News*, Oct. 6, 1951, pp. 11–13.

Gilchrist, J. D. F. 1924. A protozoal parasite *Chloromyxum thyrsites* sp. n. of the Cape sea-fish, the snoek (*Thyrsites atun*, Euphr.). *Trans. Roy. Soc. South Africa*, **11**: 263–273.

Johnston, T. H., and J. B. Cleland. 1910. The haematozoa of Australian fish. I. *J. Roy. Soc. New South Wales*, **44**: 406.

Margolis, L. 1953. Milkiness in lemon sole fillets. *Fish. Res. Bd. Canada, Ann. Rept. Pac. Biol. Sta. for 1953*, p. 158 (mimeo.).

Matsumoto, K. 1954. On the two new myxosporidia, *Chloromyxum musculoliquefaciens* sp. nov. and *Neochloromyxum cruciformum* gen. et sp. nov., from the jellied muscle of swordfish, *Xiphias gladius* Linne and common Japanese sea-bass, *Lateolabrax japonicus* (Temmink et Schlegel). *Bull. Japanese Soc. Sci. Fish.*, **20**(6): 469–478.

M'Gonigle, R. H., and A. H. Leim. 1937. Jellied swordfish. *Fish. Res. Bd. Canada, Atl. Prog. Rep.*, No. 19, pp. 3–5.

Thompson, W. F. 1916. A note on a sporozoan parasite of the halibut. *Rept. Comm. Fish. British Columbia* for 1915, pp. 127–129.

Willis, A. G. 1949. On the vegetative forms and life history of *Chloromyxum thyrsites* Gilchrist and its doubtful systematic position. *Australian J. Sci. Res.*, (Ser. B), **2**: 379–398.

Study of Red Halophilic Bacteria in Solar Salt and Salted Fish: I. Effect of Bacto-Oxgall[1]

By H. P. Dussault
Fisheries Research Board of Canada
Technological Station, Grand River, Gaspe County, Que.

ABSTRACT

Bacto-oxgall has shown a differential effect on the red halophilic bacteria commonly found in solar salt and in contaminated salted fish. *Pseudomonas salinaria* was inhibited by low oxgall concentrations while *Sarcina littoralis* tolerated high ones. When this differentiating test is applied to a larger number of strains it will be possible to determine if Bacto-oxgall can be used as the basis of a selective medium to distinguish one genus from the other. The clearing of bacterial suspensions and microscopical examination have shown that the inhibition of *Ps. salinaria* was produced by the lysis of the cells. The inhibition of *Ps. salinaria* was effective and rapid. It was not affected by pH variations but was reduced by the presence of proteins. When compared similarly to other bile products, Bacto-oxgall was found to have the same inhibitory power as sodium taurocholate. Bacto-oxgall, diluted to the equivalent of fresh bile, was also found to be three times more effective than fresh cod bile. From the evidence gathered it was concluded that the inhibition of *Ps. salinaria* by Bacto-oxgall is due to a mechanical disruption of the bacterial cytoplasm.

INTRODUCTION

It is generally accepted that the red halophilic bacteria commonly found in solar salt and in fish cured with solar salt are extremely resistant to most of the commonly used bactericides and disinfectants. Consequently, red discoloration will continue to cause serious losses to the salted fish industry as long as unsterilized solar salt is utilized or until an effective bactericide is found. Although a large number of substances have been tried, none has yet been found effective for the elimination of the causative organisms or recommended for industrial application. Hess and Gibbons (1942) have claimed some success in eliminating red halophilic bacteria by the use of commercial disinfectants and preservatives. But their results could not be reproduced by Castell and Mapplebeck (1952) who attributed the divergency to the use of different testing techniques and concluded that "the resistance of red halophiles to common disinfectants is greater than has been indicated by previous tests." More recently, Boyd and Tarr (1954) experimenting with a series of inhibitors and antibiotics found that none of the substances tested caused any important reduction of the reddening of salted fish.

It has long been known that bile acids and their salts are toxic to some types of bacteria. Thus, the lysis of gonococcus has been reported by Maino (1933), that of pneumococci of types I, II and III by Sturdza (1938), that of cholera and other vibrios by Hasegawa and Nakamoto (1939) and finally, that of tubercle bacilli and other acid-resistant bacteria by Valette and Liber (1941). On the

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other hand, Pangalos (1932) observed that both Shiga and Flexner strains of the dysentery bacillus were able to live for long periods of time in pure bile and Fuller (1938) pointed out that 30 strains of *Aerobacter aerogenes* showed no cultural reaction when grown in a suitable medium containing 1 and 5% bile or bile salts. With certain other organisms, it has been observed that bile and bile salts could produce growth stimulation. Boas and Neumuller (1930) have shown that the growth of *Saccharomyces cerevisiae* was accelerated with small concentrations of bile salts while it was reported by Takagi (1952) that bile had a growth-promoting effect on typhoid and paratyphoid bacteria.

Although a review of available literature has not disclosed any study of the effect of bile or bile salts on halophilic bacteria, Anderson and Hart (1934) have reported that the susceptibility of pneumococci to lysis by bile salts was increased by the presence of NaCl. The present investigation deals with the effect of a bile salt product, Bacto-oxgall, on the red halophilic bacteria found in contaminated solar salt and salted fish. It also includes a study of certain factors affecting the observed inhibition by the bile product and an attempt at explaining the mechanism of the inhibitory action.

EXPERIMENTAL METHODS

Organisms

The two pure cultures used were obtained from the collection of Dr. A. G. Lochhead of the Department of Agriculture, Ottawa. One was *Pseudomonas salinaria* Harrison and Kennedy and the other *Sarcina littoralis* Poulsen. These two species are always found present in reddened salt cod and in solar salt, although the latter species occurs to a lesser extent. Cadiz solar salt was also used as a source of red halophiles because it was shown by analysis to contain the two types mentioned above. This salt was obtained from a bulk shipment to one of the local merchants. At the time it was received, the sample had a total count of over 4 million red halophiles per gram.

Culture Medium

The culture medium used throughout was a modified skim milk–salt agar whose preparation has been described by Dussault and Lachance (1952). The agar medium, the nutrient brine without the skim milk, and the pure brine were all made to contain 20% NaCl, expressed as the number of grams of C.P. NaCl added to 100 ml. of medium or of distilled water. The reaction of the medium was so adjusted that the resulting pH was approximately 7.4, within the range that has been reported and observed to be the optimum for the organisms under test.

Bacto-oxgall

The Bacto-oxgall used was a Difco dehydrated product which, when diluted to a 10% concentration (W/V), is equivalent to fresh bile. In that concentration it was readily soluble in 20% brine. The present work had been planned to include for comparison other bile products such as cholic acid, desoxycholic acid, sodium desoxycholate and sodium taurocholate. However, on account of the insolubility

of most of these products in 20% brine, the study was restricted mainly to Bacto-oxgall. Sodium taurocholate and cod bile, which are both soluble in brine, were briefly studied for comparison purposes only. For each experiment, a fresh 1% solution was always prepared with 20% brine, from which successive dilutions were made.

Procedures Used

The effect of Bacto-oxgall on the red halophilic bacteria was evaluated by two types of tests. First was the immersion method which consisted in inoculating a few drops of a heavy suspension of the test organism into tubes containing oxgall serially diluted with 20% brine. After given periods of contact time and periodic shaking, one large loopful was removed from each tube and transferred to the surface of skim milk–salt agar plates for survival tests. The highest dilution showing complete inhibition was considered as the limiting one. However, no record was made of partial inhibition as indicated by slower and poorer development of the inoculum when compared with the control experiment.

The second technique, the agar dilution method, consisted in inoculating one loopful of a suspension of the test organisms onto skim milk–salt agar medium containing varying concentrations of Bacto-oxgall. Then, after incubation, the highest dilution at which inhibition occurred was recorded. The effect of Bacto-oxgall on the survival of red halophiles was also followed quantitatively by application of the "drop plate" method as described by Dussault (1954). In this method drops of appropriate dilutions of the two pure cultures and of the solar salt were added to plates of skim milk–salt agar containing varying concentrations of oxgall. After a proper incubation period, the colonies on the plates were counted, from which the total numbers of bacteria were calculated. The results obtained were expressed as percentage of the total count recorded on the control plates where oxgall had been omitted.

EXPERIMENTAL RESULTS

Effect of Bacto-Oxgall

qualitative evaluation. In this first set of tests, the effect of varying oxgall concentrations was evaluated by inoculating 4 to 5 drops of a heavy suspension of actively growing cultures of *Sar. littoralis* and *Ps. salinaria* into tubes containing 5 ml. of the serially diluted oxgall solutions. The dilutions used ranged from 1 to 1000 parts per million (p.p.m.). Immediately after inoculation, the tubes were thoroughly agitated to produce an effective distribution of the inoculum and kept for 24 hours at room temperature before testing for survival. The plates for the survival tests were incubated at 37°C. and although growth in control plates usually appeared after 6 days, incubation was continued during an extra 6 days for added insurance against slow development and bacteriostasis. According to the results obtained, *Ps. salinaria* was inhibited by 100 p.p.m. whereas *Sar. littoralis* tolerated 1000 p.p.m.

In a similar manner, a second series of tests was carried out for determining more exactly the limiting inhibitory oxgall concentrations. Thus *Ps. salinaria*

was tested with diminishing oxgall concentrations of smaller intervals and *Sar. littoralis* with increasing concentrations. Results showed that *Ps. salinaria* was inhibited by 57 p.p.m. but not by 50 p.p.m. With *Sar. littoralis*, a 5% oxgall solution did not produce any inhibition although a longer incubation period was required to show survival of the test organism.

By the agar dilution method, oxgall was added to skim milk–salt agar in such amounts as to produce concentrations ranging from 0.03 to 1%. One large loopful of a heavy suspension of the test organisms was transferred onto the surface of the dried plates. After an incubation period of 12 days at 37°C., visual observations were made for determining survival. Results of several replicate tests showed that *Ps. salinaria* was inhibited by 0.05% oxgall and *Sar. littoralis* by 0.3%.

QUANTITATIVE EVALUATION. For this series of tests the effect of oxgall added to the agar medium to give concentrations varying from 20 to 1000 p.p.m. was evaluated quantitatively by the "drop plate" method. Appropriate dilutions of the pure cultures *Ps. salinaria* and *Sar. littoralis* and of the contaminated solar salt were prepared and added dropwise onto the surface of the medium. After allowing the plates to dry they were incubated at 37°C. for 12 days, then the total counts were recorded. By comparing these counts with those obtained from the control plates, the percentage of survival was calculated. Figure 1 illustrates the results obtained for one series of such counts, and shows that the growth of *Sar. littoralis* is somewhat stimulated by the low oxgall concentrations from 20 to 100 p.p.m. At 1000 p.p.m. it is only slightly affected since approximately 98% of the original total count is recorded. Inhibition of *Ps. salinaria* is initiated by the low oxgall concentrations and is completed at 500 p.p.m. An almost similar

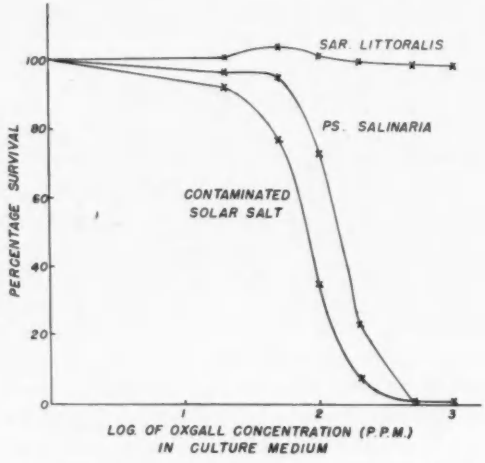

FIG. 1.—The effect of Bacto-oxgall on *Sar. littoralis*, *Ps. salinaria* and on the red halophiles in solar salt, by the drop plate method.

pattern is followed by the red halophiles present in contaminated solar salt, which are almost completely inhibited at 500 p.p.m. However a very small percentage, less than 1%, resists the action of 1000 p.p.m. of oxgall. Also microscopical examinations have shown that cultures of the resistant colonies were coccus forms apparently belonging to the genus *Sarcina*.

Lysis of *Ps. salinaria* by Bacto-Oxgall

While testing the effect of Bacto-oxgall on *Ps. salinaria* by the immersion method, it has been observed that soon after the inoculum had been added to the oxgall solution with effective shaking, immediate clarification of the suspension took place leaving a perfectly clear solution, slightly pink in colour due to released bacterial pigments. With the proper oxgall concentrations this lysis was immediate and so complete that the inhibitory concentration could be readily determined by simple visual observation. This fact has been confirmed when survival tests showed that the observed lysis always indicated death of the test organism. An illustration of the lysis phenomenon appears in Fig. 2, showing both *Sar. littoralis* and *Ps. salinaria* suspended in respective series of tubes containing varying oxgall dilutions. It is clearly evident that *Sar. littoralis* remains unaffected by contact with the three oxgall concentrations. On the other hand, *Ps. salinaria* is completely lysed at 1000 and 100 p.p.m., but is not affected by 10 p.p.m. oxgall.

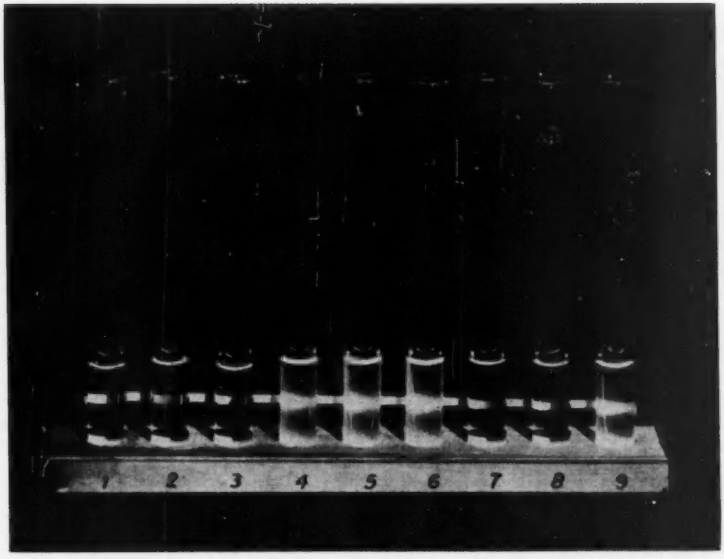

Fig. 2.—The effect of Bacto-oxgall on *Sar. littoralis* and *Ps. salinaria* by the immersion method. *1*, 2 and 3, uninoculated control tubes of 20% brine containing 1000, 100 and 10 p.p.m. Bacto-oxgall respectively. *4*, *5*, *6*, same as *1*, *2*, *3* respectively, but inoculated with *Sar. littoralis*. *7*, *8*, *9* same as *1*, *2*, *3* respectively, but inoculated with *Ps. salinaria*.

The lysis of *Ps. salinaria* by Bacto-oxgall was further studied by making microscopical examinations of the test culture after varying periods of contact with an oxgall solution containing the minimum inhibitory concentration, 57 p.p.m. On account of their great sensitivity to changes in osmotic pressure, true halophiles are ordinarily stained with great difficulty. In order to avoid immediate plasmoptysis, smears must be prepared with brine solutions of high salinity and, consequently, the salt crystals formed by evaporation have to be removed before staining. After various attempts and modifications the best results have been obtained with the following procedure: The smear is desalted and fixed simultaneously by immersing the slide in a 2% acetic acid solution during 5 minutes. The slide is dried without washing, then it is stained for 3 minutes with a fresh solution of gentian violet. Figure 3 illustrates clearly the lytic action

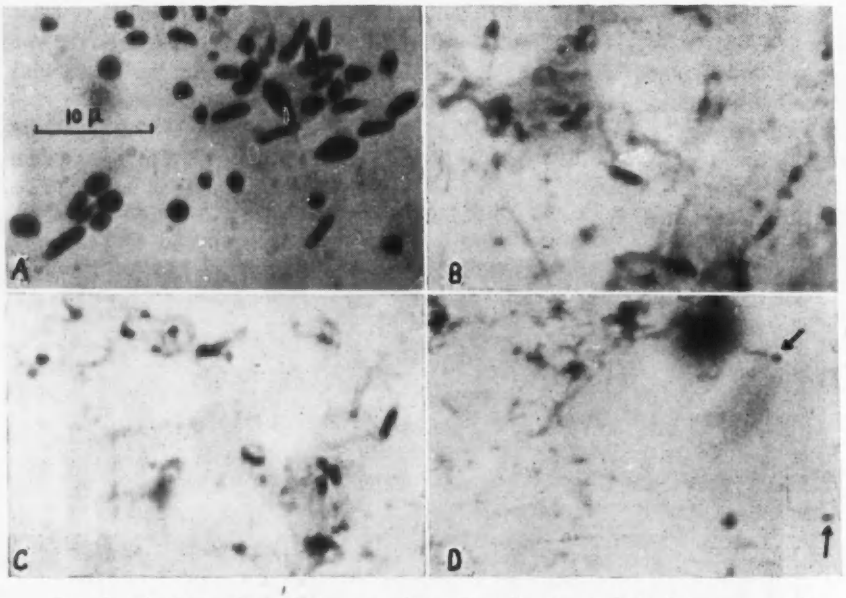

FIG. 3.—The lytic effect of Bacto-oxgall on *Ps. salinaria*. A. Actively growing culture, untreated, showing pleomorphic forms. B. Culture after 10 minutes contact with oxgall solution. C. Culture after 30 minutes contact with oxgall solution. D. Culture after 60 minutes contact with oxgall solution, showing a few nuclei (arrows) in a mass of cytoplasmic debris from lysed cells.

of Bacto-oxgall on *Ps. salinaria* after varying periods of contact time. The sequence of photomicrographs shows that, as the contact time increases, the number of whole cells diminishes and larger amounts of cytoplasmic debris are formed. After one hour, practically all the cells have been lysed and only small round bodies are left. Although no special nuclear stain was used, these bodies have been identified as bacterial nuclei.

Factors Affecting the Inhibitory Action of Bacto-Oxgall

In an effort to elucidate the mechanism by which *Ps. salinaria* is inhibited by Bacto-oxgall, the effect of such factors as time of contact, pH and addition of protein materials was investigated. An additional aim was to find out if these factors influenced the action of oxgall in a way similar to that of other surface active agents.

Effect of Contact Time

The time of contact between oxgall and the bacterial cells was the first factor investigated in order to determine the speed and efficacy of the mechanism by which *Ps. salinaria* is lysed. Table I indicates the contact time required for varying oxgall dilutions to produce complete inhibition by the immersion method. It is shown that, at 100 p.p.m. oxgall concentration, *Ps. salinaria* is inhibited

Table I.—Contact time required for the inhibition of *Ps. salinaria* by Bacto-oxgall.

Oxgall conc. in p.p.m.	No inhibition	Inhibition
50	>24 hours	....
57	2 hours	3 hours
66	30 minutes	1 hour
80	2 minutes	5 minutes
100	10 seconds	30 seconds

almost immediately and that the 30 seconds required is barely sufficient to insure a complete dispersion of the inoculated cells throughout the oxgall solution. As the oxgall concentrations diminish, the contact time necessary to produce inhibition increases gradually up to the 3-hour mark where a minimum inhibitory concentration of 57 p.p.m. oxgall is reached. Concentrations under this limit will not inhibit *Ps. salinaria* even if the contact time is increased considerably more than 24 hours.

Effect of pH

The effect of varying pH on the inhibitory power of oxgall was investigated to determine if its action is similar to that of other surface active agents. Varying oxgall dilutions were prepared with brines that had been brought to pH 5, 7 and 9 with the appropriate amount of 1N HCl and NaOH. *Ps. salinaria* was inoculated into each series and its survival after 24 hours contact time was tested by the immersion method. Results indicate that variations in pH do not affect the inhibitory power of Bacto-oxgall in any way (Table II). Although only a

Table II.—The effect of pH on the inhibitory action of Bacto-oxgall on *Ps. salinaria* after 24 hours contact time. (+ indicates survival, – indicates inhibition.)

Oxgall conc. in p.p.m.	pH		
	5	7	9
0	+	+	+
50	+	+	+
57	–	–	–
66	–	–	–
80	–	–	–

limited pH range was used, it is important to emphasize the wide total range and also the small range intervals of oxgall dilution over which changes in pH did not alter the minimum oxgall concentration necessary to inhibit *Ps. salinaria.*

EFFECT OF ADDED PROTEINS

The effect of varying concentrations of four different protein sources on the inhibition of *Ps. salinaria* by Bacto-oxgall was studied. Bacto–skim milk, Bacto-peptonized milk, blood serum and gelatin were selected. They were diluted with 20% brine to give concentrations ranging from 0 to 2%. After adding to each of these protein solutions quantities of oxgall to produce eleven concentrations ranging from 50 to 1000 p.p.m., drops of the *Ps. salinaria* suspension were inoculated and the inhibitory power of oxgall was determined by the immersion method as previously described. Table III represents the minimum oxgall concentrations at which the test organism was completely inhibited after 24 hours of contact time.

TABLE III.—The effect of various protein sources on the inhibition of *Ps. salinaria* by Bacto-oxgall. (Data represent minimum oxgall concentrations in p.p.m. causing complete inhibition.)

Protein conc. %	Protein sources			
	Peptonized milk	Gelatin	Skim milk	Blood serum
0	57	57	57	57
0.1	57	57	100	100
0.2	57	66	125	125
0.5	57	80	166	200
1.0	66	80	200	333
2.0	66	80	333	500

It is clearly indicated that peptonized milk and gelatin, even when present in a 2% concentration, exert only a limited effect on the inhibitory power of oxgall. At this protein level, the minimum inhibitory concentration was shifted from 57 p.p.m. to 66 p.p.m. of oxgall for peptonized milk and to 80 p.p.m. for gelatin. On the other hand, blood serum and skim milk showed a much greater protective action which increased somewhat proportionately to their concentration. It has been observed that while 2% solutions of the latter two protein sources formed opaque suspensions, peptonized milk and gelatin produced clear solutions.

COMPARISON WITH OTHER BILE PRODUCTS AND DETERGENTS

As mentioned earlier, most of the other bile products tried were insoluble in 20% brine and consequently they could not be compared with Bacto-oxgall. Such was the case with cholic acid, desoxycholic acid and sodium desoxycholate. However, sodium taurocholate dissolved in 20% brine as easily as Bacto-oxgall itself, producing a perfectly clear and colourless solution. Its effect on *Ps. salinaria* was studied and it was found to have the same inhibitory power as oxgall, with the minimum effective concentration at 57 p.p.m. after 24 hours contact time. Also, fresh bile extracted from cod produced a clear solution when diluted with the brine and was found to inhibit *Ps. salinaria* at a minimum concentration of

1660 p.p.m., which is approximately equivalent to 166 p.p.m. when cod bile is converted to its dry weight content.

The action of Bacto-oxgall was compared with that of some of the more widely used detergents. Roccal, Aerosol O.T. and Tween 80 were selected to represent respectively the cationic, anionic and non-ionic compounds. Roccal inhibited *Ps. salinaria* at 10 p.p.m. after an immersion time of 24 hours but not after 1 hour. Aerosol O.T. was totally insoluble in 20% brine while Tween 80 was found to produce slight growth activation even when used in concentrations as high as 1%.

DISCUSSION AND CONCLUSIONS

The results reported in the present work have shown that the *Ps. salinaria* strain studied is inhibited by 57 p.p.m. Bacto-oxgall when tested with the immersion method and by 500 p.p.m. with the agar dilution method. The fact that these minimum inhibitory concentrations, determined by the two testing methods, differ by a factor of 9 is undoubtedly due to the presence of the 5% skim milk in the agar medium. It has also shown that, when tested by the immersion method, the *Sar. littoralis* strain will tolerate more than 5% Bacto-oxgall. However, by the agar dilution method, inhibition is produced by the presence of 0.3% oxgall. At that concentration it has been observed that the skim milk of the solid medium has started to coagulate and it is probable that the protein constituents of the medium have become altered in such a way that they can no longer support the growth of *Sar. littoralis*. Consequently, the failure to grow reported in this particular test would be more appropriately referred to as bacteriostasis rather than inhibition.

The quantitative evaluation of the effect of oxgall on the red halophilic bacteria present in contaminated solar salt has revealed that, for that particular sample, the large proportion is inhibited by 500 p.p.m. oxgall and thus seems to be affected in the same manner as the pure *Pseudomonas* strain. At 1000 p.p.m. oxgall, less than 1% of the original total count remains unaffected. Microscopical examinations have shown that cultures of the surviving colonies were coccus forms, probably *Sarcina*, since Gibbons (1936) has already reported that all the red halophilic coccus forms isolated from salt fish, various salts and salting establishments belonged to the genus *Sarcina*.

It is evident that the significance of the oxgall tolerance as a distinguishing test must be interpreted with limitation until a larger number of strains has been assayed. The results of such tests have been gathered and are published in a subsequent paper (Dussault 1956). It has been possible to establish with greater certainty that the differential effect of oxgall may serve as a means of distinguishing *Ps. salinaria* from *Sar. littoralis* and that Bacto-oxgall may be used as the basis of a selective medium. Also, the results so far obtained indicate striking differences in the physiological and cytological constitution of the two typical red halophiles commonly found in solar salt and associated with the reddening of salted codfish.

The inhibition of *Ps. salinaria* by the lysis of the bacterial cell has been clearly demonstrated both by the clearing of the suspension of the inoculum when

brought in contact with the oxgall solution and by microscopical examination. Photomicrographs of *Ps. salinaria* treated with oxgall have shown that the cytoplasm of the bacterial cell was completely disrupted, leaving in the mass of debris the nuclei unchanged or only slightly altered. A similar observation has been made by Cutinelli (1949) while studying the lysis of different strains of pneumococcus with ox bile.

The reported results have shown that the lysis visually observed coincides with inhibition as indicated by the survival tests, and that it is effected rapidly after relatively short periods of contact time. Complete inhibition of *Ps. salinaria* by the minimum effective concentration of oxgall, 57 p.p.m., occured after 2 to 3 hours of contact time. Longer contact periods with slightly lower concentrations never produced any inhibition, indicating a strictly definite limit. For dilutions of oxgall in excess of the minimum inhibitory concentration the time factor did not seem to have any significance since inhibition was almost instantaneous. With 100 p.p.m. oxgall a 30-second period was sufficient to produce inhibition, just long enough to insure an effective dispersion of the cells of the inoculum in the oxgall solution.

The hydrogen ion concentration has been known to modify disinfectant action by affecting both the test organism and the disinfectant itself. With surface active agents the cationic compounds tend to become more efficient bactericides in alkaline solutions and less efficient in acid solutions, with the reverse situation holding true for the anionic compounds (Gershenfeld and Milanick, 1941). Although bile and bile salts are known to lower interfacial tensions as demonstrated by Dascher (1952), the results here described have shown that oxgall does not act in a way similar to that of other surface active agents since its toxic action was not affected by changes in pH.

It is universally agreed that the presence of organic matter lowers the efficiency of most bactericides depending on the concentration, the reactivity and the absorbability of the bactericide itself. A similar effect is noticed with surface active agents. In the present work, it has been shown that the inhibitory action of Bacto-oxgall was greatly diminished by the presence of the skim milk making up the solid medium. For the inhibition of *Ps. salinaria* by the agar dilution method, the minimum oxgall concentration was nine times that required by the immersion method. It has further been observed that varying degrees of protective action were obtained depending on the protein sources tested, blood serum and skim milk being more effective than gelatin and peptonized milk. Therefore it may be concluded that the protective action of added proteins is inversely proportional to their solubility and that the presence of suspended particles is responsible for the reduced inhibitory action of oxgall against *Ps. salinaria*.

The comparison of the effect of Bacto-oxgall with that of other bile products has produced some interesting results. Besides the bile salts, Bacto-oxgall contains other constituents such as mucin and pigments, fats, fatty acids and soaps, cholesterol, lecithin and inorganic salts, whose total sum makes up approximately 42% of the dehydrated product. However, it has been shown that

Bacto-oxgall and sodium taurocholate inhibit *Ps. salinaria* at identical concentrations. Also, when a solution of Bacto-oxgall equivalent to fresh bile was compared with fresh cod bile for the inhibition of *Ps. salinaria*, the former was approximately three times more effective than the latter. But it has been reported by Hammarsten (1904) that the only bile acid present in cod bile is cholic acid conjugated with taurine. Consequently, both these comparisons indicate that the combination of bile products present in Bacto-oxgall is much more effective than sodium taurocholate alone for the inhibition of *Ps. salinaria*.

For the purpose of explaining the inhibitory action of Bacto-oxgall its effect on *Ps. salinaria* was compared with that of some well-known surface active agents. The addition of Tween 80 in concentrations as high as 1% produced appreciable growth activation of the test organism. There is a possibility that this non-ionic surface active agent might be used advantageously as a stimulant for the cultivation of red halophilic bacteria in the same manner as for the submerged growth of the tubercule bacillus as reported by Dubos (1947). The cationic detergent Roccal produced an inhibitory effect somewhat similar to that of oxgall, but it proved much more effective since only 10 p.p.m. was required to produce inhibition of *Ps. salinaria*. The latter results are in complete disagreement with those of Castell and Mapplebeck (1952) who reported that Roccal was wholly ineffective even when used in concentrations up to 3%. It is probable that the use of different strains and different testing methods is responsible for such divergent results.

An explanation of the toxic effect of bile salts and bile acids on bacteria has been proposed by several workers. Anson (1939) mentioned the possibility that the bile salts were simply biological detergent molecules whose specific structures were of secondary importance. If an excess was added to bacteria, denaturation of the enzyme proteins probably occurred. More recently, however, Stacey and Webb (1947) have concluded that there was no general relationship between the bacteriostatic activity of bile salts and the depression of the surface tension of the medium. This was consequently interpreted as showing that the toxic action of the bile salts is due to a direct mechanical breakage or some surface components of the cells. Also in accordance with this interpretation are the results of Brodie and Shepherd (1949) whose electron microscope studies have revealed that bile salts alter the permeability of the cell-wall of susceptible bacteria, facilitating the entry of electrolytes and resulting in swelling and disruption of the cytoplasm. The evidence brought forth in the present study seems to confirm this latter theory in offering an adequate explanation for the inhibition of *Ps. salinaria* by Bacto-oxgall.

SUMMARY

1. Low concentrations of Bacto-oxgall inhibit *Ps. salinaria* and high ones are tolerated by *Sar. littoralis*.

2. The inhibition of *Ps. salinaria* occurs by the lysis of the bacterial cells as indicated by clearing of the suspension and by microscopical examination.

3. The action of Bacto-oxgall against *Ps. salinaria* is effective and rapid. It

is not affected by pH variations. The presence of proteins exerts a protective effect against its inhibitory power.

4. Bacto-oxgall has the same inhibitory power as sodium taurocholate, and, when diluted to the equivalent of fresh bile, is three times more effective than fresh cod bile.

5. The inhibition of *Ps. salinaria* by Bacto-oxgall is due to a mechanical disruption of the bacterial cytoplasm.

ACKNOWLEDGMENTS

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REFERENCES

ANDERSON, A. B., AND P. D'A. HART. 1934. *Lancet*, **II**: 359–60.
ANSON, M. L. 1939. *J. Gen. Physiol.*, **23**: 239–46.
BOAS, F., AND G. NEUMULLER. 1930. *Arch. Microbiol.*, **1**: 35–59.
BOYD, J. W., AND H. L. A. TARR. 1954. *Fish. Res. Bd. Canada, Pacific Prog. Rep.*, No. 99, pp. 22–24.
BRODIE, J., AND W. SHEPHERD. 1949. *J. Gen. Microbiol.*, **3**: 74–79.
CASTELL, C. H., AND E. G. MAPPLEBECK. 1952. *J. Fish. Res. Bd. Canada*, **9**: 377–87.
CUTINELLI, C. 1949. *Riv. ist. sieroterap. ital.*, Sez. prima **24**: 157–67.
DASCHER, G. F. 1952. *Science*, **116**: 660–63.
DUBOS, R. J. 1947. *J. Exptl. Med.*, **85**: 9–22.
DUSSAULT, H. P., AND R. A. LACHANCE. 1952. *J. Fish. Res. Bd. Canada*, **9**: 157–63.
DUSSAULT, H. P. 1954. *J. Fish. Res. Bd. Canada*, **11**: 261–66.
1956. *J. Fish. Res. Bd. Canada*, **13**: 195–199.
FULLER, J. E. 1938. *Proc. Soc. Exptl. Biol. Med.*, **38**: 507–10.
GERSHENFELD, L., AND V. E. MILANICK. 1941. *Am. Jour. Pharm.*, **113**: 306–26.
GIBBONS, N. E. 1936. *J. Biol. Bd. Canada*, **3**: 70–76.
HAMMARSTEN, O. 1904. *Hoppe-Seyl. Z.*, **43**: 109.
HASEGAWA, S., AND T. NAKAMOTO. 1939. *Japan J. Exp. Med.*, **17**: 139–49.
HESS, E., AND N. E. GIBBONS. 1942. *J. Fish. Res. Bd. Canada*, **6**: 17–23.
MAINO, M. 1933. *Bol. soc. ital. biol. sper.*, **8**: 1289–91.
PANGALOS, G. E. 1932. *Compt. rend. soc. biol.*, **110**: 678–80.
STACEY, M., AND M. WEBB. 1947. *Proc. Roy. Soc. London*, **B 134**: 323–37.
STURDZA, S. A. 1938. *Compt. rend. soc. biol.*, **129**: 410–12.
TAKAGI, A. 1952. *Hukuoka Acta Med.*, **43**: 510–521.
VALETTE, G., AND A. LIBER. 1941. *Compt. rend. soc. biol.*, **135**: 849–51.

Study of Red Halophilic Bacteria in Solar Salt and Salted Fish: II. Bacto-oxgall as a Selective Agent for Differentiation[1]

By H. P. Dussault
Fisheries Research Board of Canada
Technological Station, Grand River, Gaspe County, Que.

ABSTRACT

A simple method, based on oxgall tolerance, is proposed for differentiating red halophilic bacteria commonly found in solar salt and discoloured salted codfish. Tests carried out on 18 strains isolated from various sources have shown that the rod forms are inhibited by low concentrations of Bacto-oxgall and that the coccus forms tolerate relatively high ones. Bacto-oxgall can thus be used as the basis of a simple selective medium. This test has been found useful for the isolation, purification and partial identification of unidentified strains and also for determining the relative proportions of the two main types of red halophilic bacteria present in solar salt and salted fish samples.

INTRODUCTION

On account of their bacteriolytic effect on certain types of bacteria, bile acids and their salts have long been used as selective agents and have also served as the basis of selective media. McKinney (1934) and Evans (1936) employed Bacto-oxgall in bile solubility tests for differentiating pneumococci from the bile-insoluble streptococci. Using a slightly modified testing procedure with the same group of organisms, Greey (1939) reported excellent results. Littman (1947) described an agar medium in which Bacto-oxgall served for the isolation of pathogenic fungi. The differentiation of pneumococci from other microorganisms that produce greenish discoloration on blood agar by the use of bile salts was reported by Harris and McClure (1942). More recently, Brodie (1948) reported that bile salts could be used for the differential inhibition of coliform bacilli and rough variants of intestinal pathogens.

In a preceding paper, Dussault (1956) has reported that the oxgall solubility test could be successfully applied to the red halophilic bacteria commonly found in solar salt and responsible for the reddening of salted codfish. Comparing one pure culture of *Pseudomonas salinaria* Harrison and Kennedy with one pure culture of *Sarcina littoralis* Poulsen, it was shown that low concentrations of Bacto-oxgall completely inhibited the former while high concentrations were tolerated by the latter. In order to propose this oxgall test for differentiating one type from the other, it had to be applied to a larger number of strains. The present paper deals with the results of the application of the oxgall test to several strains of red halophilic bacteria obtained from various sources.

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EXPERIMENTAL METHODS

The material and methods employed in the present paper are essentially the same as outlined in the preceding one (Dussault, 1956). The oxgall solubility tests were carried out on eighteen pure cultures of red halophilic bacteria obtained from Dr. N. E. Gibbons of the National Research Council, Ottawa, and represented members of the coccus and rod groups. The rod group contained cultures identified as *Ps. salinaria, Ps. cutirubra,* strains of *Bacterium halobium* and some unidentified species. The coccus group was made up of *Sar. littoralis, Micrococcus morrhuae,* strains of *Sar. morrhuae* and some unidentified cultures. However, the variable cultural characteristics and the uncertain taxonomy of the organisms in the above collection make it possible that many are identical. All the cultures grew very satisfactorily on the skim milk–salt agar employed and the oxgall tolerance tests were always carried out from actively growing cultures.

EXPERIMENTAL RESULTS

The experimental work consisted in studying the effect of Bacto-oxgall on all the test organisms by both the immersion and the agar dilution method for determining either their oxgall tolerance or the minimum concentration at which inhibition occurred. From actively growing cultures on slopes of skim milk–salt agar heavy bacterial suspensions were prepared in sterile 20% brine. For the immersion method, 4 to 5 drops of these suspensions were added into tubes containing 5 ml. brine to which Bacto-oxgall had been added in different concentrations. The tubes were effectively swirled to insure complete dispersion of the inocula and kept at room temperature for 24 hours. The survival tests were carried out by transferring one large loopful from each tube onto the surface of skim milk–salt agar plates which were then incubated at 37° C.

For the agar dilution method, one large loopful of the heavy inocula was transferred onto the surface of skim milk–salt agar medium containing different oxgall concentrations. After drying, all the plates were incubated at 37° C. for at least 12 days, even though good growth appeared after only 4 days on the control plates where oxgall was omitted.

The results obtained are summarized in Tables I and II. They show that the test organisms can be separated into two definite groups according to their oxgall tolerance and that this differentiation is in complete agreement with that based on their morphology. All nine members of the first group (Table I) are

TABLE I.—Effect of Bacto-oxgall on the rod group red halophilic bacteria. Values (parts per million, p.p.m.) represent the minimum oxgall concentrations causing complete inhibition.

Organism	Source	Immersion method	Agar dilution method
		p.p.m.	*p.p.m.*
Ps. salinaria	From salt and salted fish by Lochhead	57	500
Ps. cutirubra	From salted hides by Lochhead	50	<400
B. halobium 6.31.1	From Dead Sea by Elazari-Volcani	66	600
B. halobium 6	From gut of hermit crab by Zahl	66	600
B. halobium	Source unknown, from Mossel, Utrecht	80	600
4 Unidentified	From solar salt by Castell	50	500
N-1 "	From Newfoundland salt	50	500
Sc-2 "	From salted sausage casing	57	600
A-22 "	From Norway, source not indicated	50	500

rod forms. When tested by the immersion method, they were all inhibited by 80 p.p.m. Bacto-oxgall or less. By the agar dilution method, 600 p.p.m. was the minimum oxgall concentration which caused complete inhibition of all the members of the group tested.

The second group (Table II), made up of nine coccus forms, had a much higher oxgall tolerance. The results obtained by the immersion method show

TABLE II.—Effect of Bacto-oxgall on the coccus group red halophilic bacteria. Values (percentages) represent the minimum oxgall concentrations causing complete inhibition (*indicates no inhibition).

Organism	Source	Immersion method	Agar dilution method
		%	%
Sar. littoralis	From salt and salted fish by Lochhead	5*	0.3
Sar. morrhuae Volcani	From Dead Sea, by Elazari-Volcani	5*	0.5
Sar. morrhuae 9.5	From Dead Sea, by Elazari-Volcani	0.5	0.3
M. morrhuae Dead Sea	From Dead Sea, by Elazari-Volcani	5	0.3
Sar. morrhuae	Source unknown, from Mossel, Utrecht	0.5	0.3
Sar. morrhuae Delft	Brought from Delft by Elazari-Volcani	5*	0.3
N-5 Unidentified	From Newfoundland salt	5*	0.3
A-7 "	From Norway, source not indicated	0.5	0.3
A-14 "	From Norway, source not indicated	5*	0.3

that five out of the nine strains tolerated more than 5% Bacto-oxgall, one was inhibited by 5% and the remaining three were inhibited by 0.5%. When tested by the agar dilution method, all the strains were inhibited by 0.3% Bacto-oxgall or more.

The oxgall differentiation test was further applied to a group of unidentified laboratory cultures that had been isolated from various samples of solar salts and reddened codfish. In all, 28 cultures of red halophiles were tested and separated into rod and coccus group according to their oxgall tolerance.

Microscopical examinations of these cultures for morphology has confirmed the accuracy of the grouping.

DISCUSSION AND CONCLUSIONS

During the isolation, purification and identification of the red halophilic bacteria found in solar salt and reddened salted codfish, the major difficulty encountered by several workers consists in the pleomorphic character of the *Pseudomonas* species. The intermediate coccoid forms observed during the growth cycle of the organisms have been attributed to such factors as age of the culture, nature and salt content of the medium, incubation temperature and staining technique (Harrison and Kennedy, 1922). These coccoid forms are often confused with the true cocci of the *Sarcina* species and even microscopical examinations do not offer an adequate criterion for identification and for establishing the purity of *Pseudomonas* cultures.

Dussault (1956) has already reported that the presence of protein material exerts a protective action against the inhibitory power of Bacto-oxgall. This explains why testing by the agar dilution method requires a much higher oxgall concentration than by the immersion method to produce the same inhibition

of the test organisms. The results also show that slight variations in oxgall tolerance exist between members of the rod group. When a greater number of tests are made on a larger number of strains, it might be possible to differentiate, for example, *Ps. cutirubra* which is inhibited by 50 p.p.m. oxgall from *Ps. salinaria* which is inhibited by 57 p.p.m.

On the other hand, all the members of the coccus group tolerate substantially higher oxgall concentrations than are required to cause complete inhibition of all the rod forms. When tested by the immersion method, five out of the nine strains are not affected by the presence of 5% Bacto-oxgall. However, by the agar dilution method, inhibition of practically all the strains is produced by the presence of 0.3% oxgall. In this case, the presence of protein material does not show any reduction of the inhibitory power of oxgall. On the contrary, inhibition occurs at a much lower oxgall concentration than when tested by the immersion method. Anson (1939) has reported that a solution of beef methemoglobin was denatured by the addition of 0.25% sodium taurocholate and also that bacterial growth did not appear in a solution of hemoglobin containing 1% of a commercial detergent, Duponol PC. These findings seem to support the conclusion reached in the author's preceding paper (Dussault, 1956) that the presence of 0.3% oxgall causes denaturation of the protein constituents of the culture medium to such an extent that it can no longer support the growth of the test organisms. Consequently, the inhibition of the members of the coccus group, as determined by the agar dilution method, would be more appropriately referred to as bacteriostasis.

The results obtained establish with certainty that major differences exist in the physiological and cytological constitution of the two groups of red halophilic bacteria. Therefore, the oxgall tolerance test may serve as a means of distinguishing one from the other and Bacto-oxgall may be used as the basis of a selective medium. The immersion method offers a rapid means of differentiation by the simple visual observation of the lysis phenomenon which has been shown to indicate complete inhibition of the members of the rod group (Dussault, 1956).

Although the limiting oxgall concentration for the complete inhibition of members of the rod group is 80 p.p.m. by the immersion method and 600 p.p.m. by the agar dilution method, it is recommended, for practical purposes, to use 100 and 1000 p.p.m. Bacto-oxgall respectively. At these concentrations, members of the coccus group are not inhibited. Also this would eliminate the taking into account of such factors as strain variability, contact time between the inoculum and the oxgall solution and errors introduced during the preparation of oxgall dilutions.

It is expected that the oxgall differentiation test will be of great value for the primary grouping of red halophilic bacteria. It will also greatly simplify the isolation of pure strains of the coccus form from mixed cultures and will remove the confusion occasioned by the observation of pleomorphic coccoid forms in cultures of strains belonging to the rod group. The oxgall test has already been used in this laboratory for isolating and purifying unidentified cultures of red halophiles obtained from various sources. It has also served to determine, by

quantitative studies, the relative proportions of the two main types of red halophilic bacteria present in discoloured salted codfish and in solar salt samples.

SUMMARY

The effect of Bacto-oxgall on eighteen strains of red halophilic bacteria isolated from various sources has been studied. All the members of the rod group are inhibited by low oxgall concentrations and all those of the coccus group tolerate relatively high concentrations. Inhibition by oxgall can thus be used for differentiating one group from the other and Bacto-oxgall can serve as the basis of a simple selective medium.

This test has been found most useful for isolating and purifying unidentified strains and also for determining the relative proportions of the two main types of red halophilic bacteria present in discoloured salted codfish and in solar salt samples.

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REFERENCES

ANSON, M. L. 1939. *J. Gen. Physiol.*, **23**: 239–46.
BRODIE, J. 1948. *J. Gen. Microbiol.*, **2**: 1–7.
DUSSAULT, H. P. 1956. *J. Fish. Res. Bd. Canada*, **13**: 183–194.
EVANS, A. C. 1936. *J. Bact.*, **31**: 423–37.
GREEY, P. H. 1939. *J. Inf. Dis.*, **64**: 206–207.
HARRIS, A. H., AND G. Y. MCCLURE. 1942. *J. Lab. Clin. Med.*, **27**: 1591–92.
HARRISON, F. C., AND M. E. KENNEDY. 1922. *Trans. Roy. Soc. Canada*, Sect. V, **16**: 101–160.
LITTMAN, M. L. 1947. *Science*, **106**: 109–111.
MCKINNEY, R. A. 1934. *J. Bact.*, **27**: 373–96.

A Note on the Production of Nitrite from Hydroxylamine by Some Heterotrophic Bacteria[1]

By C. H. Castell and E. G. Mapplebeck
Fisheries Research Board of Canada
Technological Station, Halifax, N.S.

ABSTRACT

Small amounts of nitrite slowly accumulated in cod fillets that had been dipped in a solution of hydroxylamine. Cultures of *Pseudomonas* isolated from fish, as well as two species of *Proteus* and one species of *Microbacterium*, were able to convert hydroxylamine to nitrite when grown in a nutrient broth.

INTRODUCTION

In the course of some tests comparing the preservative effect of sodium nitrite on cod muscle with that of hydroxylamine, a rather unexpected observation was made. During storage, small quantities of nitrite gradually accumulated in hydroxylamine-treated cod (Table I). Nitrite did not form when hydroxylamine was added to sterile fish muscle; nor did it form in normally contaminated muscle in the absence of hydroxylamine.

Table I.—The nitrite content of cod fillets stored at −1° C. Prior to storage one lot had been dipped into a 0.2% solution of sodium nitrite, the second into a 0.2% solution of hydroxylamine and the third was left as a control.

Days in storage	Nitrite content		
	Nitrite-dipped fillets	Hydroxylamine-dipped fillets	Control fillets
	p.p.m.	*p.p.m.*	*p.p.m.*
0	155	0	0
4	129	3	0
7	95	4	0
9	104	8	0
11	96	17	0
14	98	15	0
18	89	38	0

These observations suggested that the nitrite had its origin in the hydroxylamine and that it was formed through the activity of microorganisms. The work described here was an effort to determine whether any heterotrophic bacteria, and more especially those isolated from fisheries products, are capable of converting hydroxylamine into nitrite.

As hydroxylamine is either bactericidal or bacteriostatic in very low concentrations it was first necessary to determine the maximum amount that could

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be incorporated in a culture broth without inhibiting growth of the bacteria to be tested. The literature on this subject is somewhat indefinite. As far back as the last century Kitasato (1890) found that 0.05% entirely prevented the growth of *Cl. tetani*, *Cl. chauvii* and *Cl. septicum*. Jensen (1944) has shown that 0.005% hydroxylamine hydrochloride inhibited the growth of food poisoning staphylococci. In more recent work (Jensen, 1954) he found that eight different bacterial species were inhibited by 6 to 50 parts per million (p.p.m.) of hydroxylamine.

Tarr (1945, 1953) studied the effect of hydroxylamine on the growth of bacteria in nutrient broth, fish digest broth and in minced Pacific cod muscle. From 0.005 to 0.05% caused transient to permanent growth inhibition on the ten organisms tested, including cultures of *Micrococcus*, *Flavobacterium* and *Achromobacter*. From 0.0024 to 0.024% delayed the development of the normal mixed flora in minced cod stored at 0°C. Hydroxylamine "retarded the development more strongly in acid than in neutral or alkaline media, but the effect of pH was neither as pronounced nor as universal as in the case of inhibition due to nitrite."

Fedorov (1946) and Novak and Wilson (1948) found that traces of hydroxylamine (1 to 2 μg. per ml.) were extremely toxic to *Azotobacter*. Gray and Lambert (1948) added hydroxylamine to nutrient agar and found that 0.0125% was required to inhibit *S. typhi*, *E. coli*, *P. vulgaris* and hemolytic streptococci and 0.0083% to inhibit *Staph. aureus* and *B. subtilis*. This would seem to indicate that concentrations somewhere between 1 and 250 p.p.m. of hydroxylamine are required to inhibit bacterial growth and that the amount may vary with different species of bacteria.

The work described in this paper was therefore divided into two parts: first, the determination of the limiting concentrations of hydroxylamine permitting growth of the organisms to be tested; second, finding whether any of the cultures are capable of converting hydroxylamine into nitrite.

EXPERIMENTAL METHODS

The medium used consisted of a sterilized solution containing 0.5% each of Bacto yeast extract, beef extract and peptone. In a few tests with *Clostridia* it was replaced by a modification of Brewer's medium (Reed and Orr, 1941).

These broths were autoclaved in 9-ml. amounts in small screw-capped bottles and the hydroxylamine, which is decomposed by autoclaving, was added separately to the cooled, sterile broth.

The hydroxylamine used was obtained as the hydrochloride from Eastman Kodak. A 1% solution in water was prepared, the pH of which was adjusted to 6.6 with sodium hydroxide. One ml. of various dilutions of this stock solution was added to each of the previously autoclaved bottles of broth. The calculations for the dilutions are in terms of hydroxylamine and not the hydrochloride.

The cultures tested consisted of pure cultures of identified organisms and bacteria isolated from fish and most were identified as far as their genera. Inoculations were made by adding one drop of a 24- to 48-hour culture that had been incubated at 25°C. The test cultures were also incubated at 25°C.

Quantitative nitrite and hydroxylamine were both measured by Blom's

method, modified by Endre, as outlined by Novak and Wilson (1948). Where only qualitative tests were required, the same procedure was adapted to the use of spot plates instead of the colorimeter.

EXPERIMENTAL

Part I. Bacterial Tolerance for Hydroxylamine

In the first tests, broths containing 0, 10, 20, 30, 50, 80 and 100 p.p.m. of hydroxylamine were inoculated with the pure cultures to be examined. (The concentrations referred to here and throughout this paper indicate the amount of hydroxylamine present at the time inoculations were made. In the uninoculated controls there was a slow decrease during storage and in the inoculated cultures the decrease was often quite rapid.) These were examined daily for a period of 32 days to determine if and when growth occurred. The tests were repeated three times. The results are summarized in Tables II and III. In general the results indicated that members of the *Enterobacteriaceae* could withstand higher concentrations of hydroxylamine than the *Pseudomonas*, *Micrococcus* and *Flavobacterium* that were tested. The few cultures of *Bacillus* and *Microbacterium* could also withstand greater concentrations than the few cultures of *Sarcina* and

Table II.—The maximum initial concentrations of hydroxylamine permitting growth in broth cultures for various identified stock cultures and other organisms isolated from fisheries products.

Name	Number of cultures tested	Hydroxylamine
		p.p.m.
A. aerogenes	2	50–100
A. cloacae	2	50–100
E. coli	4	50–100
E. communior	2	50–100
Sal. enteritidis	1	50–80
Prot. vulgaris	3	50–100
Prot. morganii	1	50–100
Ser. marcescens	2	80–100
Alc. metalcaligenes	1	50–100
Alc. ammoniagenes	1	50–100
Ps. fluorescens	8	20–30
Ps. fragi	2	20–30
Ps. putrefaciens	4	20–30
Sar. lutea	2	20–30
Mic. varians	4	20–50
Mic. citreus	2	20–30
Mic. epidermidis	2	20–30
Mic. conglomeratus	2	20–30
Mic. candidus	2	20–30
Mic. caseolyticus	1	20–30
Mic. aurentiacus	1	20–30
Microb. piscarium	3	30–100
B. subtilis	1	50–100
B. mycoides	1	50–100
Cl. butyricum	5	10–50
Cl. sporogenes	3	10–50
Flavobacter from fish	9	20–80
Pseudomonas from fish	40	20–30
Yellow halophiles	8	100

TABLE III.—The number of days before growth was visible in ten broth cultures initially containing 0 to 100 p.p.m. of hydroxylamine.

Organism	Number of days for visible growth					
	0 p.p.m.	20 p.p.m.	30 p.p.m.	50 p.p.m.	80 p.p.m.	100 p.p.m.
A. aerogenes	1	1	1	2	2	3
E. coli	1	1	2	5	5	6
Ser. marcescens	1	1	2	3	3	5
Microb. piscarium	2	2	3	7	7	10
Mic. varians	2	2	5	10	10	15
B. subtilis	1	3	3	7	10	32
Ps. fluorescens	1	1	0	...	...	...
Ps. putrefaciens	1	3	5	...	...	...
Flav. solare	2	3	5	10	10	...
Sar. lutea	2	3	...	15	...	...

Clostridium tested. From these results it was concluded that under the conditions of these tests a concentration of 20 to 30 p.p.m. of hydroxylamine permits growth of most of the bacteria encountered in this work.

TOLERANCE OF YEASTS, MOULDS, *Actinomycetes* AND CERTAIN HALOPHILIC BACTERIA FOR HYDROXYLAMINE

Some common, powdery white, unidentified cultures of *Actinomycetes*, when tested under the same conditions as the bacteria, were inhibited by 20 to 30 p.p.m. of hydroxylamine, although a few isolated colonies developed only after a long incubation period in a broth containing 50 p.p.m.

When malt extract agar was used in place of the peptone–yeast–beef broth many common saprophitic moulds grew readily and formed spores when the hydroxylamine concentration was 200 to 250 p.p.m. These included one or more cultures of *Oospora lactis* and *Monilia sitophila*, and unidentified species of *Alternaria, Cladosporium, Trichoderma, Penicillium, Aspergillus* and *Stemphylium*. The growth was a little slower and a little more scanty at the highest concentrations but after 5 days' incubation all the plates were covered with moulds and there was abundant spore formation. Strains of *Hormodendrum* and *Spicaria* grew on plates containing 100, but not 200, p.p.m. A *Helminthosporium* was inhibited by all concentrations above 50 p.p.m.

Saccharomyces cerevisiae and some unidentified yeasts grew on malt extract agar plates containing up to 200 p.p.m. of hydroxylamine.

No tests were made to determine how much more than 200 or 250 p.p.m. of hydroxylamine was required to inhibit the growth of these yeasts and moulds. Considering the rate and the amount of growth that did occur at these levels, especially with *Penicillium*, it would appear that it was far from the maximum.

It was also observed that many of the halophilic bacteria isolated from spoiling salt cod could withstand double and treble the amounts of hydroxylamine that inhibited the growth of the non-halophilic species.

PART II. BACTERIA PRODUCING NITRITE FROM HYDROXYLAMINE

Cultures of bacteria were inoculated in peptone–yeast–beef broth containing approximately 25 p.p.m. hydroxylamine. These were incubated at 25°C. and

tested daily during 12 days for the presence of nitrite. Nitrite was never found present immediately after inoculations were made. In most cases where it did develop, it was present after 24 and 48 hours but frequently disappeared on further incubation. With the identified cultures the following observations were made (the figures in parentheses indicate the number of cultures examined):

Nitrite found present in:		Nitrite not present in:	
Proteus vulgaris	(6)	*Escherischia coli*	(6)
Proteus morganii	(1)	*Aerobacter aerogenes*	(3)
Microbacterium piscarium	(6)	*Aerobacter cloacae*	(2)
Unidentified *Pseudomonas*	(63)	*Serratia marcescens*	(2)
Pseudomonas fragi	(3)	*Salmonella enteritidis*	(1)
		Achromobacter candicans	(2)
		Unidentified *Achromobacter*	(5)
		Flavobacterium solare	(1)
		Flavobacterium proteus	(2)
		Flavobacterium marinum	(2)
		Unidentified *Flavobacterium*	(11)
		Bacillus subtilis	(3)
		Bacillus mycoides	(1)
		Sarcina lutea	(2)
		Micrococci sp.	(11)
		Pseudomonas putrefaciens	(7)
		Unidentified *Pseudomonas*	(23)
		Unidentified halophiles	(17)

DISAPPEARANCE OF NITRITE FORMED IN THE HYDROXYLAMINE BROTH

Seventy unidentified cultures of green and blue-green *Pseudomonas* isolated from fish and fish plant equipment were inoculated into hydroxylamine broth and also into ordinary nitrate broth. Periodically these were tested for the presence of nitrite in the hydroxylamine broth and for the presence of nitrite and nitrogen gas in the nitrate broth. From the results obtained, these organisms could be divided into four groups.

1. Twenty-three cultures reduced nitrate to nitrite and nitrogen. All but two of these cultures produced nitrite from hydroxylamine, but the nitrite so formed later disappeared.
2. Twenty-eight cultures produced neither nitrite nor nitrogen from nitrate but did produce nitrite from hydroxylamine. The nitrite so formed was still present when the experiment terminated after 2 weeks' incubation.
3. Four cultures produced nitrite but not nitrogen from nitrate and did not produce nitrite from hydroxylamine.
4. Fifteen cultures produced neither nitrite nor nitrogen from nitrate and did not produce nitrite from hydroxylamine.

This would seem to indicate that the disappearance of nitrite formed from the hydroxylamine is the result of the nitrite-reducing enzyme that is present in these particular organisms. Confirmatory results were obtained (Table IV) when pure cultures of eight other bacteria were similarly tested.

TABLE IV.—The production, and in some cases the subsequent disappearance, of nitrite in hydroxylamine broth inoculated with pure cultures of bacteria. It can also be observed that with certain bacteria the hydroxylamine disappears without the formation of nitrite.

Organism	In hydroxylamine broth					Nitrite reducers
	Nitrite present at			Hydroxylamine present at		
	1 day	3 days	12 days	6 days	12 days	
Ach. candicans	−	−	−	−	−	−
B. subtilis	−	−	−	−	−	−
Mic. citreus	−	−	−	+	+	−
Flav. solare	−	−	−	+	+	−
Pseudomonas No. 78	+	+ ?	−	−	−	+
Pseudomonas No. 84	+	+	+	−	−	−
Prot. morganii	+	+	+	−	−	−
Prot. vulgaris	+	−	−	−	−	+
Control flasks	−	−	−	+	+	...

SUMMARY

It was observed that cod muscle dipped in a solution of hydroxylamine developed small amounts of nitrite during storage. Because this was not found with sterilized muscle similarly treated with hydroxylamine nor in fish in the absence of hydroxylamine, it was concluded that the origin of the nitrite was hydroxylamine and that it was formed through the activity of microorganisms.

By inoculating various fish-spoiling bacteria into a peptone–yeast–beef broth containing approximately 25 p.p.m. hydroxylamine, it was shown that many cultures of *Pseudomonas* were able to convert the amine, at least in part, into nitrite. Furthermore, those cultures which were shown to contain a nitrite-reducing enzyme later reduced the nitrite formed from the hydroxylamine.

It was shown that certain members of the *Proteus* and *Microbacterium* also possessed this characteristic of producing nitrite from hydroxylamine. It was not found in other cultures tested.

The significance of the heterotrophic bacteria apparently oxidizing hydroxylamine to nitrite has not been discussed. This will have to await the results of a quantitative study of hydroxylamine, nitrite and nitrate metabolism of these bacteria.

REFERENCES

FEDOROV, M. V. 1946. *Compt. rend. acad. sci., U.R.S.S.*, **52**: 81–84 (*Chem. Abstr.* 1947, **41**: 2111).

GRAY, J. D. A., AND R. A. LAMBERT. 1948. *Nature*, **162**: 733–734.

JENSEN, L. B. 1944. *Bact. Rev.*, **8**: 161–188.

1954. Microbiology of Meats. 3rd Ed. The Garrard Press, Champaign, Ill., pp. 48–49.

KITASATO, S. 1890. *Z. Hyg. Infectionskrankh.*, **9**: 97–102.

NOVAK, R., AND P. W. WILSON. 1948. *J. Bact.*, **55**(4): 517–524.

REED, G. B., AND J. H. ORR. 1941. *War Med.*, **1**: 493–510.

TARR, H. L. A. 1945. *J. Fish. Res. Bd. Canada*, **6**(4): 349–350.

1953. *J. Fish. Res. Bd. Canada*, **10**(2): 69–75.

Spoilage of Fish in the Vessels at Sea: 3. The Value of Nitrite Ice and Nitrite Dips for the Preservation of Gutted Fish in the Hold of the Vessel[1]

By C. H. Castell and G. K. Gunnarsson
Fisheries Research Board of Canada
Technological Station, Halifax, N.S.

ABSTRACT

Sodium nitrite, used as a 1% dip, or as a 0.1% solution made into flake ice, has been used with gutted cod and haddock in trawlers under normal fishing conditions. The dipping was more effective in preserving the fish than storing them in the ice containing nitrite. Dipping added about 4 days to the keeping time of the fish in the boats at sea.

There appears to be little or no advantage in using nitrite either as a dip or in ice with fish that are properly iced and not stored longer than 6 days.

INTRODUCTION

In the previous paper of this series (Castell, MacCallum and Power, 1956) it was pointed out that, with some exceptions, the conditions permitting the rapid growth of bacteria on fish during stowage at sea, rather than the extent of initial contamination, determine the rate of spoilage in the hold. For this reason proper icing by which the fish are rapidly chilled, properly drained, and held at temperatures close to 0°C., is the most important measure for controlling quality of fish in the trawlers.

But even efficient icing has its limitations. Experience has shown that the maximum stowage time for well-iced cod and haddock in our Canadian Atlantic trawlers is from 7 to 14 days, depending upon the size of the fish, season of the year, location of the banks, and more particularly on whether the fish were feeding or not.

As well as decreasing the temperature, there are other means of slowing bacterial growth. Most chemical food preservatives are bacteriostats and they might well be used to supplement refrigeration in the vessels. Carbon dioxide has been used with some success for increasing the keeping time of chilled meat and fish, but it requires a special type of gas-tight hold construction. A wide variety of other preservatives is also available and could be used if the experiments were kept to a relatively small scale. But as it was intended to treat large amounts of fish that would reach the normal commercial markets, it was deemed wise to stay within the provisions of the Canada Food and Drugs Act and Regulations, which include sodium nitrite as one of the few materials (the others being sodium or potassium nitrate, salt, sugars, vinegar, wood smoke, spices, and alcohol) that are permissible for use on fresh fish.

Tarr and his associates at the Pacific Fisheries Experimental Station in a long series of Progress Reports and scientific papers (1939a, 1939b, 1939c, 1940a,

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1940b, 1940c, 1940d, 1941a, 1941b, 1942, 1944, 1947, 1948) first demonstrated the preservative value of nitrite on fish. Since then it has been used extensively for the preservation of fresh fillets in Eastern Canada. It was also shown by Tarr and Sunderland (1940a, b, c) that sodium nitrite can be used as a preservative by incorporating it into the ice used for chilling the fish. Unlike the dip, the use of "nitrite ice" for preserving fish has not become a commercial practice. One reason has been the difficulty of getting an even distribution of the nitrite in the ice because of its tendency to become concentrated in the liquid phase during freezing.

The chief purpose of this portion of the work was to determine if sodium nitrite is as useful a preservative for gutted fish as it has proved to be for fillets, and to do the investigation on a large scale under conditions as they now exist in commercial practice. The first problem was to find a method of producing the necessary amounts of nitrite ice with an even distribution of the salt within the ice. The second was to compare the value of dips with that of ice as a means of applying the nitrite to the fish.

Other preservatives, more effective than nitrite, may at some later date be made legal for use on fresh fish in Canada. Many of the problems involved in their application to gutted fish at sea will be similar to those met with in the use of nitrite. It is hoped that the following data will be useful beyond their direct application to this particular compound.

It was intended that these tests would be made with both haddock and cod, but principally with haddock, to keep the tests as uniform as possible with those in previous papers in this series. However, the type of fish caught in the nets, not the preconceived intentions, determined this point. During most of these trips, haddock were extremely scarce and cod were abundant, therefore most of the tests were made with cod.

EXPERIMENTAL PROCEDURES

It was found that the difficulty of producing a uniform distribution of nitrite in large quantities of ice could be solved by the use of "drum freezers". With these machines a thin film of water is frozen on the surface of a refrigerated rotating steel drum, from which it is scraped off in the form of small thin flakes. A stock nitrite solution was prepared by dissolving 10 lb. (4.5 kg.) of industrial grade sodium nitrite in 44.5 lb. (20.2 kg.) of tap water, followed by filtration through a large Büchner funnel. This stock solution was used within 24 hr. after it had been made up. Tests showed that by adding 12.5 cc. of this solution per minute to the water supply feeding one of the smaller freezers, a flake ice was produced that contained approximately 0.1% sodium nitrite. The solution was fed in automatically by means of a burette attached to a constant-level reservoir of stock nitrite solution. Most of the ice used in these tests was made with a York drum freezer having a capacity of about 250 lb. (115 kg.) per hour. It might be worth mentioning that although a solution of sodium nitrite is not corrosive, in the older machines it did cause leakage by reducing the oxides that had formed in the rusty portions of joints and gaskets.

During each of four trips to sea, made during the months of February, July, August, and September, similar fish were divided into two groups and iced down in opposite pens using 0.1% nitrite ice for one lot and ordinary flake ice for the controls. At the time the vessel was being discharged 20 to 30 fish were taken from each of these pens and re-iced in their respective ices in large fish boxes for further study. Some fish were taken from each lot and tested immediately to determine the effect of the nitrite on the quality during their period at sea. During these and other trips to sea, in addition to the fish iced down in the pens smaller amounts of fish were iced and stowed directly in large boxes.

A third group of fish was dipped in nitrite solution. The 1% solution used was prepared by dissolving the contents of a previously weighed 2-lb. (0.9-kg.) package of industrial grade sodium nitrite in 20 gal. (91 liters) of sea water in a clean half-puncheon. The gutted fish were washed as usual in the wash box and then forked into the puncheon containing the preservative solution until this was full. After an immersion time which ranged from 30 sec. to 3 min., the fish were forked into the hold and iced down with normal flake ice. As the boat was being discharged representative fish were taken both for immediate testing and for further observations on their keeping time on being re-iced in boxes.

For most of the quantitative determinations of nitrite, the method outlined by Dyer (1945) was used. In some of the field checks where the colorimeter was not available, the semi-quantitative spot plate method of Spurway (1938) was employed. Trimethylamine determinations were made by Dyer's method (1945; 1950).

The concentration of nitrite used in the ice was approximately 0.1% as recommended by Tarr and Sunderland (1940b) who stated: "ice containing 0.5 per cent of sodium nitrite causes the treated fish to absorb rather large amounts of the salt, and for this reason in most experiments 0.1 per cent was employed". The concentrations of sodium nitrite in solutions usually recommended for preservative dips is from 0.2 to 0.4%, depending upon the size of the fish. This leaves a residue of approximately 200 parts per million (p.p.m.) in the fish after draining them. These figures are based on results obtained from dipping fillets and do not necessarily apply to gutted fish. For this reason, some preliminary tests were made to determine the residual nitrite content of dipped gutted fish.

RESULTS OF PRELIMINARY LABORATORY EXPERIMENTS

Effect of Nitrite Concentration on Residual Nitrite in Dipped, Gutted Fish

Medium-sized gutted haddock were dipped for 2 min. in concentrations of nitrite ranging from 0.1 to 1.0%. The temperature of the solution was approximately 5°C. and the fish had been chilled overnight in ice. The dipped fish were stored for 24 hr. at 0°C. (in ice) and then filleted. The results of nitrite determinations (Table I) show that a concentration of 1% can be used without having a residue in the flesh that would exceed the maximum (200 p.p.m.) allowed by the Canada Food and Drugs Act and Regulations.

In order to determine the effect of the size of the fish on the amount of nitrite taken up from the solution, small, medium, and large cod were simul-

TABLE I.—The amount of nitrite in fillets cut from medium-sized haddock 24 hr. after the gutted fish had been dipped in solutions containing 0.1 to 1% sodium nitrite and stored at 32° F.

Concentration of nitrite dip	Average nitrite concentration in the fillets
%	*p.p.m.*
0.1	14
0.2	17
0.3	18
0.4	22
0.5	38
0.6	56
0.7	69
0.8	75
0.9	138
1.0	150

taneously dipped into a 0.8% nitrite solution for 2 min. and then stored for 24 hr. at 0°C. The concentrations of nitrite in the fillets at this time are given in Table II. These results show, as in the case of the fillets, that the size of the fish affects the amount of nitrite taken up from the solution.

TABLE II.—The concentration of sodium nitrite in the flesh of gutted scrod, market, and steak cod 24 hr. after being dipped for 2 min. in 0.8% nitrite solution.

Size of fish	Fillet number	Fillet weight	Concentration of nitrite in each fillet	Average concentration of nitrite
		g.	*p.p.m.*	*p.p.m.*
Scrod	1	160	148	
	2	170	121	
	3	180	134	133
	4	180	128	
Market	1	330	113	
	2	300	115	
	3	310	103	107
	4	290	97	
Steak	1	710	59	
	2	590	69	
	3	720	59	60
	4	490	53	

EFFECT OF NITRITE CONCENTRATION ON THE KEEPING TIME OF DIPPED, GUTTED FISH

Although the purpose of this paper was to test the use of nitrite as a preservative under normal working conditions on the trawlers, a preliminary experiment was done with gutted fish at the laboratory to get an idea of the concentration that would be effective. A group of medium-sized cod was obtained directly from the hold of a boat as it was being discharged. They had been well iced for 3 days in the boat and were all in a good state of preservation. These were divided into seven lots and dipped for 2 min. in solutions containing 0, 0.2, 0.5, 0.8, 1.0, 1.2, and 1.5% sodium nitrite. The temperature of the dip was approximately 6°C. They were then carefully iced in small pens and re-iced when necessary

during the subsequent storage period. After 3, 5, 6, 7, 10, and 11 days they were examined for signs of deterioration and measurements were made for trimethylamine from fillets cut from the fish (Table III). At 5 days there were slight spoilage odours in the gut cavity of most of the control fish; at 6 days they were distinctly sour and at 7 days they were becoming putrid.

The fish dipped in 0.2% nitrite developed very slight spoilage odours on the sixth day; they were stale but edible on the seventh day, and quite spoiled by the tenth day. The fish dipped in 0.5% nitrite spoiled almost as rapidly as those in the 0.2% solution, but they spoiled differently. This difference in the type of spoilage as the result of using a nitrite dip became more apparent where the fish had been dipped in higher concentrations. Instead of the usual stale>sour> putrid succession of odours as the fish spoiled, these nitrite-treated fish developed sweet, fruity, and sour-fruity odours. This would seem to indicate that the nitrite not only retarded spoilage but also changed the spoilage pattern in the decomposing fish. However it can be seen in Table III that nitrite retarded the development of spoilage odours for 1 to 6 days depending upon the concentration of the solution used in the dip.

TABLE III.—Odour of fillets cut from gutted cod that had been dipped in various concentrations of sodium nitrite and stored in ice up to 11 days. (These fish had previously been stored in ice at sea for 3 days before dipping.)

Days in storage	Concentration of dips						
	0%	0.2%	0.5%	0.8%	1.0%	1.2%	1.5%
3	Good	Good	Good	Good	Good	Good	Good
5	Slight spoilage	Good	Good	Good	Good	Good	Good
6	Stale or sour	Good?	Good	Good	Good	Good	Good
7	Going putrid	Stale or sour	Slight	Good	Good	Good	Good
10	Rotten	Rotten	Sweet and fruity	Stale	Good	Good	Good
11	Rotten	Rotten	Strong, fruity	Sour, fruity	Fruity, stale	Slightly sweet	Slightly sweet

Figure 1 shows the daily increase of trimethylamine obtained from freshly cut fillets from the same fish. It can be seen that the rate of development of this particular spoilage product in the fish is retarded by increasing the concentration of nitrite in the dipping solution.

It has been shown previously that the nitrite content of dipped fillets gradually decreased during storage (Dyer, 1949; Dyer and Castell, 1949). One would expect the same to apply to gutted fish that have been treated. This is confirmed by Fig. 2, which shows the decreasing nitrite content during storage in ice for the six lots of fish described in the preceding experiment.

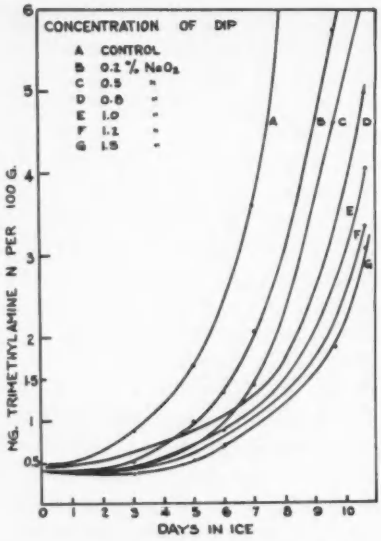

FIG. 1.—Trimethylamine curves obtained from freshly cut fillets taken from gutted cod that had been dipped in various concentrations of sodium nitrite solution and then stored in ice for 11 days.

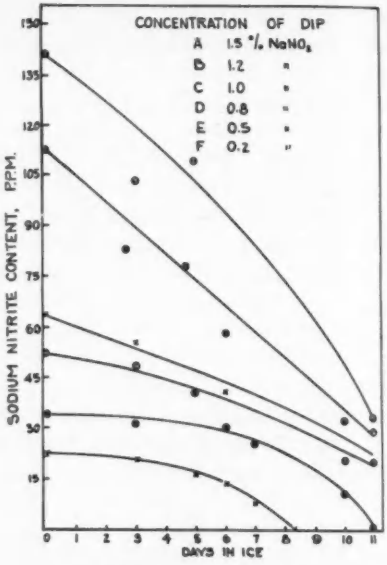

FIG. 2.—The decreasing nitrite content in the muscle of 6 lots of gutted cod that had been dipped in solutions of sodium nitrite and then stored in ice for 11 days.

EXPERIMENTS AT SEA

DIPPED FISH

In the first test 1500 lb. of large cod were dipped in 1.0% sodium nitrite before being iced in the hold and a similar amount of untreated fish was iced in the opposite pen for a control. When the boat was being discharged these fish had been in storage for 7 days. At this time all the fish were in a good state of preservation and were free from odours. The trimethylamine values[2] for 20 fish taken from each pen at this time were:

	Nitrite-treated	Controls
Average TMA	0.58	0.60
Maximum TMA	1.19	1.24
Minimum TMA	0.21	0.19

Approximately 200 lb. of fish were transferred from each lot to large boxes and kept well iced for further observation. The trimethylamine values and odours of the freshly cut fillets were:

Total days in ice	Nitrite-dipped		Control fish	
	Average TMA	Odour	Average TMA	Odour
7	0.25	None	0.23	None
11	0.65	None to slightly stale	2.45	Stale
13	0.99	None to stale	6.46	Sour
15	2.14	Stale to sour	7.83	Bad
18	12.1	Bad	17.2	Bad

[2]"Trimethylamine values" (TMA) are the number of milligrams of trimethylamine nitrogen per 100 g. of fish.

The control fish were definitely spoiling at 10 days while those dipped in the nitrite did not reach approximately the same state until about 4 days later.

The above experiment was repeated using the same amounts of large cod. At the time of discharge these fish were a day older than those in the previous test. At this time, 8 days, the control fish were still edible, but spoiling. The trimethylamine values for 20 fish taken from each pen at the time of discharge were:

	Nitrite-dipped	Controls
Average TMA	0.47	2.06
Maximum TMA	0.79	3.75
Minimum TMA	0.18	1.79

Fish from these pens were also iced in boxes for further storage studies. After 14 and 16 days in ice, fillets from 5 fish from each box were tested with the following results:

Total storage time	Nitrite-dipped			Controls		
	Av. TMA	Max. TMA	Min. TMA	Av. TMA	Max. TMA	Min. TMA
14 days	0.42	0.78	0.18	11.1	16.8	6.7
16 days	2.16	2.5	1.6	13.7	17.0	10.6

The treated fish were beginning to develop spoilage odours at 15 days, which was 7 days later than for the same condition in the controls. After another 2 weeks in ice, 28 days in all, the treated fish were still far from being spoiled from the standpoint of odours but the muscle was becoming very soft.

In the third test 3,000 lb. (1400 kg.) of large haddock were dipped in nitrite, as above, and a similar amount was left untreated for a control. Both lots were iced down in pens. At the time of discharge these fish were only 4 days in ice and could not be distinguished from each other by odour, appearance, or trimethylamine values. On further storage, 20 fish from each pen iced in boxes gave the following average trimethylamine values:

Total storage time	Nitrite-dipped	Controls
6 days	0.64	0.74
9 days	0.83	1.52

After 8 days in ice, the control fish were developing strong odours and the fillets cut from them were beginning to smell objectionable. At 9 days the untreated fish were still in a good state of preservation. At this time it was necessary to terminate the test but from these meagre results the indications are that the treated fish would outlast the controls by at least 3 or 4 days.

Nitrite-iced Fish

On two trips in the trawler 14,000 and 3,000 lb. (6400 and 1400 kg.) of cod were iced down with 0.1% nitrite ice and similar amounts used as controls. At the time of discharge from the vessel, the fish in the first test had been 7 days in the hold while those of the second test had been stored only 6 days. At this time the round fish and fillets cut from them were in a good state of preservation in both the treated and control lots. Twenty to 30 representative samples were then

taken from each pen and re-iced in large boxes at this Station, where they were examined and tested periodically. The results are shown in Tables IV and V.

TABLE IV.—First test at sea with nitrite ice. Average, maximum, and minimum trimethylamine values and odours of fillets freshly cut from fish stored in 0.1% nitrite ice and in normal flake ice up to 14 days.

Days in ice	Nitrite-treated fish				Control fish			
	Av. TMA	Max. TMA	Min. TMA	Odour	Av. TMA	Max. TMA	Min. TMA	Odour
8	1.1	1.7	0.5	Fresh?	2.0	2.3	1.6	Fresh
10	1.4	1.7	1.1	None	1.2	1.7	0.6	None
11	3.3	5.5	1.3	Slightly sour	3.3	5.1	1.8	Slightly sour
12	5.5	6.8	3.6	Sour	5.7	9.2	4.0	Sour
13	5.3	6.2	4.4	Sour	8.3	9.3	7.5	Putrid
14	13.8	17.1	7.5	Quite sour	23.9	28.9	17.1	Putrid

TABLE V.—Second test at sea with nitrite ice. Average, maximum, and minimum trimethylamine values of fillets freshly cut from fish stored up to 14 days in 0.1% nitrite ice and controls in normal flake ice.

Days in ice	Nitrite-treated fish				Control fish			
	Av. TMA	Max. TMA	Min. TMA	Odour	Av. TMA	Max. TMA	Min. TMA	Odour
6	0.6	...	...	Fresh	0.7	0.9	0.4	Fresh
9	1.8	2.6	1.3	Slightly sour	1.9	2.6	1.4	Slightly sour
12	1.6	2.4	1.4	Sour	6.7	8.8	4.0	Sour
14	3.7	3.0	4.3	Sour	9.7	12.6	8.8	Putrid

In the first test the fish had just about reached their maximum keeping period at the time the fish were being discharged. The following day the fillets were graded as being "doubtfully fresh". It can be seen by the table that both the treated and untreated fish had a very similar spoilage rate during the initial stages. There was this difference, however. After the twelfth day, when both lots were already spoiling, the control fish rapidly became very putrid while the nitrite-iced fish became increasingly sour but not putrid.

The second test gave similar results (Table V). About the ninth day both lots of fish developed slight spoilage odours. Between the twelfth and fourteenth days, the control fish became putrid. At this same period the nitrite-iced fish also became inedible from the development of various sour odours, but they did not become putrid. During this stage the trimethylamine developed more rapidly in the control fish than it did in the fish in nitrite ice.

EXPERIMENTS AT SEA WITH BOXED FISH

In addition to the tests made with the larger numbers of fish iced down in the pens in the normal manner, a series of tests was made using smaller quantities of fish iced down in large fish boxes. Each box held 25 to 30 fish and immediately after icing it was carried down into the hold for stowage.

(1) In the first test using haddock, the control fish were forked from the wash box directly into the storage box. The treated fish were forked from the wash box into a large puncheon containing 1% nitrite solution, left for 2 min., and

then iced down with ordinary flake ice in the storage box. After 8 days the control fish were beginning to spoil, and were quite sour and had a faecal odour at 12 days. A few of the treated fish had very slight odours at 12 days, and were quite "fruity" and somewhat faecal after 14 days. This treatment added 4 to 5 days to the keeping time of the fish.

(2) In the second set, using cod, one lot of fish was iced down with 0.1% nitrite ice; a second lot was dipped for approximately 2 sec. in 1% nitrite solution; a third lot was dipped for 5 min. in the same solution, and a fourth lot was left untreated. The last three were iced with normal flake ice. The curves for the average trimethylamine content for these fish are seen in Fig. 3.

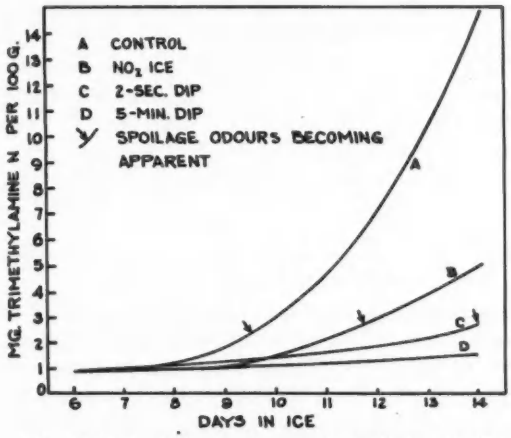

FIG. 3.—Trimethylamine curves obtained from freshly cut fillets taken from gutted cod, comparing the preservative effect of 0.1% nitrite ice with 2-sec. and 5-min. dips in 1.0% solution of sodium nitrite. These fish were all stored in large fish boxes.

Between the ninth and tenth days, the control fish were beginning to develop spoilage odours and at 12 days they were quite bad. Between the eleventh and twelfth days the fish in nitrite ice were beginning to spoil and by the fourteenth day, they were "stale", "bad", or "sour", but not putrid, although quite inedible. The dipped fish were very slightly sour at 12 days; edible but beginning to be sour or stale in the nape region at 14 days. At the end of the storage period, the fish dipped for 5 min. were slightly better than those dipped for only 2 sec. Here, the nitrite ice added about 2 days and the nitrite dip added at least 4 days to the keeping time of the fish.

(3) In the third test, also conducted with cod, a comparison was made with similar fish given the following treatments:

A. Stored in pen of vessel with ordinary flake ice (controls).
B. Stored in boxes with ordinary flake ice (controls).
C. Dipped in 1% nitrite solution and stored in pen with ordinary flake ice.
D. Dipped in 1% nitrite solution and stored in boxes with 1% nitrite flake ice.

A summary of the results obtained is given in Fig. 4. At 11 to 12 days the control fish, both in the pen and in the storage box, were beginning to spoil. Subsequent to this period the fish stored in the pen deteriorated more rapidly. The nitrite-dipped fish were beginning to spoil at 15 to 16 days but many were still edible after 18 days in ice. Once again the dip added about 4 days to the keeping time of the fish, while a combination of both nitrite dip and nitrite ice added approximately 6 days.

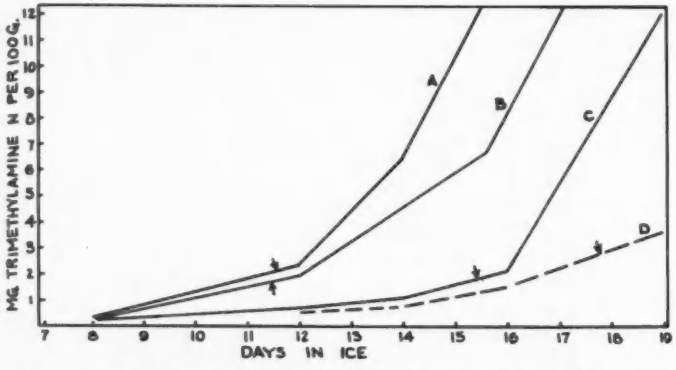

FIG. 4.—Trimethylamine curves obtained from freshly cut fillets taken from gutted cod held under conditions A, B, C, and D described in the text above. Arrows indicate times at which spoilage odours from the fillets became apparent.

NITRITE CONTENT OF FISH ICED WITH NITRITE ICE

The results show that the nitrite ice was more effective when used in boxes than when it was used in the pens. In the boxes there was a more intimate mixing of ice and fish; in the pens, where much larger amounts of fish were involved, there was always a tendency for the fishermen to pile the fish in layers 6 to 14 in. deep, separated by thinner layers of ice. Towards the bottom, where the fish were under considerable pressure, it is doubtful if there was an even flow of the melting ice-water over the surface of the fish; and it was very improbable, with fish jammed tightly against each other that the melting ice-water penetrated the gut and gill areas where the most active spoilage takes place. As this melting ice-water is the carrier of the nitrite, it would seem probable that in the pens there might be an uneven distribution of the nitrite in the fish. For this reason a comparison was made of nitrite content of fillets taken from gutted fish and nitrite-iced fish in pens and boxes, after an 8-day stowage period. In both cases 0.1% nitrite ice was used. It can be seen from Table VI that the distribution of nitrite was very much more uniform in the boxed fish. Although these data do not show whether the nitrite solution had a similar distribution in penetrating the body cavities, one might expect that the contrast might be the same or even greater.

TABLE VI.—The distribution of nitrite in the fillets from 18 boxed and 10 penned fish that were iced down for 8 days using 0.1% nitrite ice.

Nitrite range	Percentage of fillets	
	Penned fish	Boxed fish
p.p.m.		
0– 10	20	0
11– 50	0	0
51–100	20	100
101–150	20	0
151–200	0	0
201–300	40	0

After a stowage period of 9 days the nitrite content of fish that had been dipped in a 1% nitrite solution and iced in boxes with normal flake ice was compared with similar fish, undipped but iced with 0.1% nitrite ice. The average values for 5 fish were: dipped, 117 p.p.m.; nitrite-iced, 126 p.p.m. After 3 and 5 days further storage the corresponding figures were 91 and 44; 99 and 52 p.p.m. respectively.

SUMMARY AND DISCUSSION

These results indicate that under some conditions nitrite can be a useful preservative for gutted fish in the boats at sea. As far as the quality of the fish at the time of landing is concerned, there would appear to be little or no advantage in applying it to fish that are stowed in the boat for 6 days or less under conditions similar to those that existed in these tests. If used on the fish caught during the first few days of the trip, the total period during which fish of good quality may be landed can be lengthened by several days. No attempt was made during these tests to determine the effect of the nitrite applied to the dressed fish at sea on the keeping time of the fillets subsequently cut from these fish. This is a point that should be examined thoroughly before any specific recommendations are made for its actual commercial practice.

If the nitrite is incorporated into ice, by freezing a 0.1% solution in a drum-type freezer, it can be utilized with the least trouble to the fishermen in their work on the deck. This, however, does not prolong the keeping time of the fish as well as dipping the gutted fish into a 1% sodium nitrite solution immediately before icing them down in the hold.

The efficiency of the nitrite ice depends to some extent on the method of icing. When the fish and the ice were well mixed in large boxes the preservative effect was reasonably consistent and added about 2 days to the keeping time. With fish iced down in layers in the pens, the results were variable. It has been suggested that the distribution of the nitrite solution from the melting ice over the stored fish may account for this. In some instances the initial spoilage odours developed at approximately the same rate in the fish iced with nitrite ice and the ordinary ice; but the progress of the spoilage from this point through the

more offensive stages was retarded in the nitrite-iced fish. The accumulation of trimethylamine was also retarded.

For fish stored either in pens or boxes a short dip in 1% sodium nitrite solution consistently added at least 4 days to their keeping time. It would appear that the chief value of the dip over the use of nitrite in ice is that in the former the nitrite is applied not only to the outer surfaces, but also to the gut and oral cavities, where most spoilage takes place.

The amount of nitrite taken up by the fish is determined among other factors by the size of the fish. A 1% solution with dips up to 5 min. resulted in fish with a nitrite content that is permissible under the Food and Drugs Act and Regulations of Canada. It would seem advisable, however, not to use the same strength solution for very large fish and small scrod fish; a safe concentration for the small fish is likely to be less effective on the larger ones.

These experiments were performed chiefly with cod and to a lesser extent with haddock. In a few instances the fillets cut from fish dipped in the stronger nitrite solutions had a slight pink discoloration at the thinner portions, at the tail, and near the belly flap. This disappeared after a few hours standing. The effect of nitrite concentration on the discoloration of other species of fish was not investigated.

ACKNOWLEDGMENT

Sincere appreciation is given to A. L. Wood, Senior Engineer at this Station, for his generous assistance in adapting the drum freezers used in this work to the production of nitrite ice.

REFERENCES

Castell, C. H., W. A. MacCallum and H. E. Power. 1956. *J. Fish. Res. Bd. Canada*, **13**(1): 21–39.

Dyer, W. J. 1945. *J. Fish. Res. Bd. Canada*, **6**(5): 351–358.
1946. *Ibid.*, **6**(6): 414–418.
1949. *Ibid.*, **7**(8): 461–470.
1950. *Ibid.*, **7**(10): 576–579.

Dyer, W. J., and C. H. Castell. 1949. *Ibid.* **7**(9): 536–544.

Spurway, C. H. 1938. Tech. Bull. 132, 2nd Revision, "Soil Testing", Michigan State Coll. Agric. Exptl. Sta., East Lansing, Mich.

Tarr, H. L. A. 1941a. *J. Fish. Res. Bd. Canada*, **5**(3): 265–275.
1942. *Ibid.*, **6**(1): 74–89.
1944. *Ibid.*, **6**(3): 257–266.
1947. *Ibid.*, **7**(3): 101–115.
1948. *Ibid.*, **7**(4): 155–161.

Tarr, H. L. A., and P. A. Sunderland. 1939a. *Fish. Res. Bd. Canada, Pacific Prog. Rep.*, No. 39, pp. 13–16.
1939b. *Ibid.*, No. 40, pp. 14–16.
1939c. *Ibid.*, No. 41, pp. 15–16.
1940a. *Ibid.*, No. 44, pp. 16–17.
1940b. *J. Fish. Res. Bd. Canada*, **5**(1): 36–42.
1940c. *Ibid.*, **5**(2): 148–163.
1941b. *Ibid.*, **5**(3): 244–248.

Atlantic Salmon Tagged in East Coast Newfoundland Waters at Bonavista[1]

By A. A. Blair
Fisheries Research Board of Canada
Biological Station, St. John's, Nfld.

ABSTRACT

In 1940, from June 8 to June 28, 68 salmon and 386 grilse were tagged at Bonavista on the east coast of Newfoundland. The fish used were caught by commercial fishermen in salmon traps set along the coast within 3¼ miles of the lighthouse at Bonavista.

The proportion of tagged salmon recaptured during the year of tagging was 41.2%, and 1.5% in the following year. The 28 tagged salmon recaptured in 1940 were distributed as follows: Newfoundland 57.1%, mainland of Canada 39.3%, and Labrador 3.6%. The greatest distance travelled was 785 miles, and the fastest apparent rate of travel was 26.2 miles per day.

The tagged grilse returns were 36.3% in 1940 (year of tagging), 2.6% in 1941, and 0.3% in 1942. The 140 tagged grilse recaptured in 1940 were distributed as follows: Newfoundland 92.9%, and mainland 7.1%. The greatest distance travelled by recaptured grilse was 792 miles, and the fastest apparent rate of travel was 32.5 miles per day, both exceeding the highest values for salmon. The average interval between tagging and recapture (during the same year) for the grilse taken commercially on the east coast of Newfoundland was 14.3 days, on the south coast 20.2 days, and on the mainland 28.8 days.

INTRODUCTION

There are many rivers in Newfoundland almost all of which contain grilse (1-sea-year fish) in fair numbers, but only a few possess salmon (2-sea-year and older fish) in any quantity. Very little is known about the Labrador rivers in respect to the relative quantities of grilse and salmon. In the coastal net fishery salmon and grilse are caught all around the island of Newfoundland and also in Labrador. Salmon are taken in greatest quantities on the east coast of Newfoundland and in Labrador.

Generally speaking, both grilse and salmon appearing in coastal waters during the spring and summer months are maturing fish which will spawn in various rivers during the succeeding fall months. For conservation purposes it is important to know the destination of these fish and the proportion captured, and tagging gives a good indication of both.

In 1940, from June 8 to June 28, 68 salmon and 386 grilse were tagged at Bonavista on the east coast of Newfoundland. Previous publications on the tagging of salmon in Newfoundland waters consist of one by Belding and Préfontaine (1938) on the salmon of the 1937 Port aux Basques drift-net fishery and one by Belding (1940) which gives a brief account of the results of tagging operations at Port aux Basques in 1937 and 1938, and at St. Anthony in 1938.

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METHODS

The location of tagging was within a radius of approximately 1 mile from the lighthouse at Bonavista. Only 4 salmon and 1 grilse were tagged outside of this area, namely at Danson Cove which is 3¼ miles from the Bonavista lighthouse.

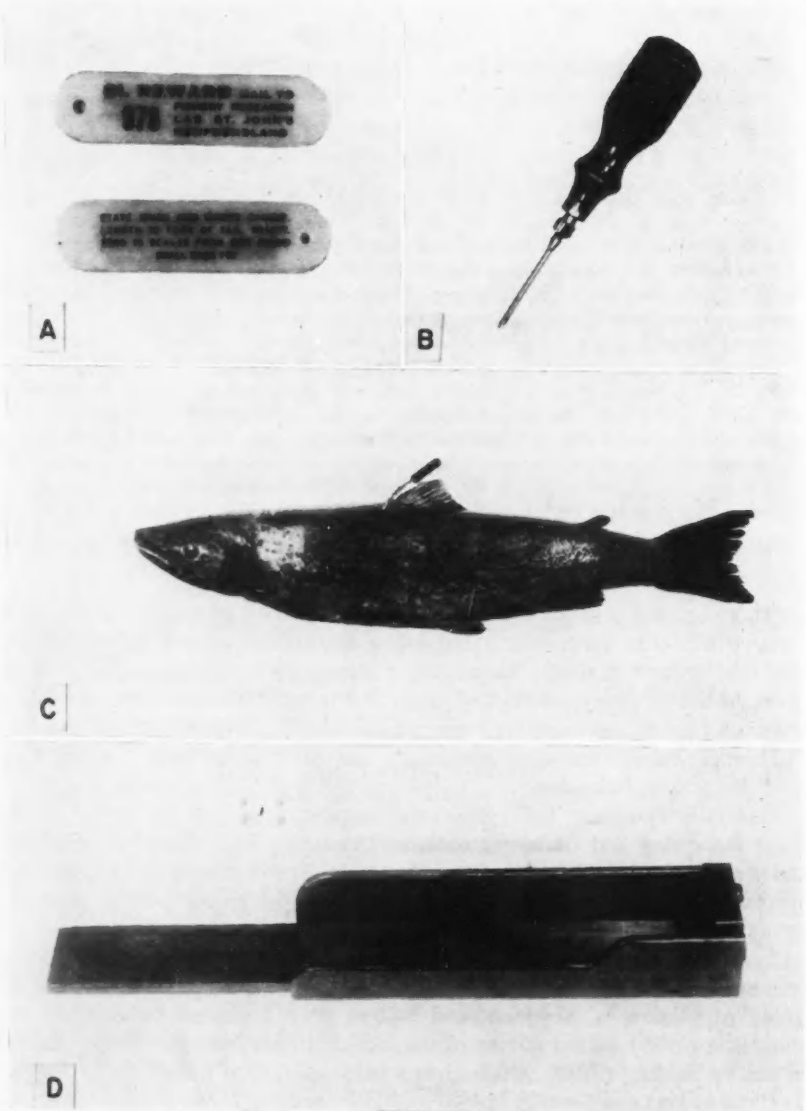

FIG. 1. A—Tag showing printing on each side; B—Hypodermic needle for putting tag wire through fish; C—Tag attached to fish; D—Measuring board with side pieces for holding fish.

The fish were purchased from the fishermen at the prevailing local price per pound, the weight being estimated. Both gill nets and traps are operated in this area, but only fish caught in traps were used for tagging because they are in good condition since they do not mesh, or mesh only when the trap is being hauled. The tagging was done in a small boat, and a cod-bag of cotton twine was used for holding the fish in the water until required. In the actual tagging operation one person held the fish on a measuring board while another attached the tag, recorded length, and took a sample of scales. The measuring board (Fig. 1D) was fitted with side boards to facilitate holding the fish. Scales were taken just above the lateral line and just anterior to the adipose fin. The tag was attached to the back of the fish immediately in front of the dorsal fin (Fig. 1C).

Each tag was in a separate scale envelope with the number of the tag written on the outside. When the tag was removed, the scale sample was put in the same envelope, and the information was recorded on the outside. The tags (Fig. 1A) were rectangular pieces of red celluloid 31 mm. long, 8 mm. wide, rounded at both ends, and with a hole in one end where a 5-inch (12.7-cm.) piece of soft nickel wire was fastened. In attaching the tag to the fish a hypodermic needle (Fig. 1B) was pushed through the back of the fish, and the wire was threaded through the needle which was then withdrawn and the wire twisted into a loop by hand. Care was taken to get the wire beneath one or more of the interspinous bones of the dorsal fin so that the tag would be firmly attached.

When these operations were completed, the tagged fish were released immediately if they were lively. The passive fish, however, were held in the water until they struggled to get away which usually took only a few seconds. The latter procedure was not very often necessary.

A reward of one dollar was offered for return of a tag. This fact and the address were printed on the tag (Fig. 1A). No additional effort was made to influence the recovery of tags.

The number of salmon tagged was small because of stormy weather, which made it difficult to tag and at times prevented fishermen from hauling their traps. In addition to the difficulty of tagging during a storm, it was found that it was hard to get salmon out of the traps in good condition for tagging at such times. When the weather became suitable for tagging, the main run of salmon was over, and salmon were scarce. The grilse run, however, was just beginning, and a fair number of grilse were tagged. The grilse run is usually several weeks later than the salmon run.

TAGGED SALMON (EXCLUDING GRILSE)

Proportion Recaptured

Of 68 salmon tagged, 29, or 42.6%, were recaptured, 28, or 41.2%, being taken in 1940 and only 1, or 1.5%, in 1941 (Table I). The tagged salmon caught in

Table I.—Recaptures in 1940 and 1941 from 68 salmon tagged in 1940.

Year of recapture	1940	1941	Total
Number of recaptures	28	1	29
Percentage of total recaptures	96.6	3.4	100
Percentage of number tagged	41.2	1.5	42.6

1940 were taken as follows: 13 in June, 7 in July, 5 in August, 1 in September, and 2 with indefinite dates. All of them recaptured in 1940 were taken before spawning, but one was caught between Sept. 10 and Oct. 16 in the vicinity of Southesk, Northwest Miramichi River, New Brunswick, in nets operated during the close season for the Canadian Department of Fisheries to obtain salmon for the government hatchery at Southesk. This salmon, along with others, was kept in a retaining pond until mature when it was stripped and released alive. Thus the number taken by the normal fishery during the open season was 27, or 39.7%.

Forty per cent exploitation does not seem enough to cause concern about overfishing, but there are several considerations which bear on the representativeness of this figure.

1. The total number tagged and recaptured was small, hence purely random error may be high.

2. There is some possibility the tag or the tagging may affect vulnerability to fisheries—either decreasing or increasing it. In this work, a tagged salmon may be less likely to take a fly, thus making the anglers' catches low.

3. The fish tagged were from the latter part of the run, June 8–28. (The time of the *salmon* run at Bonavista is usually from May 10 to June 20, with the peak around June 1). Since catching effort tends to be greatest when fish are most abundant, the rate of return of salmon tagged earlier in the season would probably have been greater.

4. Although the reward offered was large enough that almost all commercially caught tags would be returned, the same is not true of those taken by anglers. For most of the latter, the reward was not an effective inducement to return a tag; in fact some of them would not appreciate the implication that they wanted the money, and would be more apt to keep a tag as a souvenir. Thus the anglers' recaptures obtained are considered too low, both absolutely and relative to commercial recaptures.

5. Finally, since the fish tagged had already been in the fishery for a longer or shorter time (as shown by the fact they were caught in regular commercial gear) a part of their period of exposure to fishing effort was past; hence the rate of commercial recapture would tend to be too low to be representative of the complete seasonal rate of exploitation for these fish.

On the other hand, it is possible that some salmon habitually remain somewhat farther from the coast than others, and so would be less heavily represented among the group tagged.

The one salmon which was recaptured in 1941 was a kelt taken April 28 by rod on the Northwest Miramichi River in New Brunswick. The interval from one spawning to the next is usually two years in the case of salmon, and it is therefore surprising that not even one recapture was reported in 1942.

Distribution of Recaptures

The distribution of recaptured salmon taken in 1940 and 1941 is given in Table II, and Fig. 2 shows the distribution in 1940. The 28 tagged salmon recaptured in 1940 were distributed as follows: Newfoundland 57.1%, mainland

of Canada 39.3%, and Labrador 3.6%. The high proportion recaptured on the mainland and the low proportion in Labrador will come as a surprise to the fishermen and those connected with the salmon fishery on the east coast of Newfoundland, since the consensus of opinion has been that the salmon in this area are headed for Labrador. Presumably this opinion is based on the fact that salmon strike in first in the southerly sections of the coast and later and later in the more northerly sections, in which case it is the time of arrival of the first of the run which is the basis for thinking the salmon are headed for Labrador. As mentioned above it was the last part of the run at Bonavista which was tagged, and it is possible that the returns from tagging the first or the peak of the run might present a somewhat different picture of the movements of salmon from this area. Moreover, the wide dispersal of recaptures indicates that more tagging is desirable and that it should be carried out at several points along the east coast of Newfoundland and on the Labrador coast during the same year.

In 1938 salmon (and grilse?) were tagged by Belding (1940) at St. Anthony which is on the east coast of Newfoundland near the Strait of Belle Isle. Recaptures from this tagging were reported from the following areas: (1) the east coast of Newfoundland (few) as far south as White Bay, (2) the west coast of Newfoundland as far south as Bay of Islands, (3) the east coast of Labrador, and (4) the north shore of the Gulf of St. Lawrence as far west as Natashquan which is directly north of the eastern end of Anticosti Island. Unfortunately the percentage recaptures in these areas are not given, but it should be noted that area (4) is on the mainland. Salmon (no grilse) were also tagged in the Port aux Basques drift-net fishing area in 1937 by Belding and Préfontaine (1938) who found that 40% of the recaptured salmon were taken

TABLE II.—Distribution of recaptured salmon tagged at Bonavista in 1940, in actual numbers and as percentage of the total recaptures.

Area	Year of recapture				
	1940		1941	Total	
	No.	%	No.	No.	%
Newfoundland					
Labrador	1	3.6	...	1	3.4
White Bay	1	3.6	...	1	3.4
Notre Dame Bay	1	3.6	...	1	3.4
Bonavista Bay	9	32.1	...	9	31.0
Trinity Bay	1	3.6	...	1	3.4
Conception Bay	1	3.6	...	1	3.4
St. John's	1	3.6	...	1	3.4
Near Cape Ray	2	7.1	...	2	6.9
Nova Scotia	7	25.0	...	7	24.1
New Brunswick	3	10.7	1	4	13.8
Quebec	1	3.6	...	1	3.4
Total	28	100	1	29	100
Summary					
Newfoundland–Labrador	1	3.6	...	1	3.4
East coast Newfoundland	14	50.0	...	14	48.3
West coast Newfoundland	2	7.1	...	2	6.9
Other provinces	11	39.3	1	12	41.4

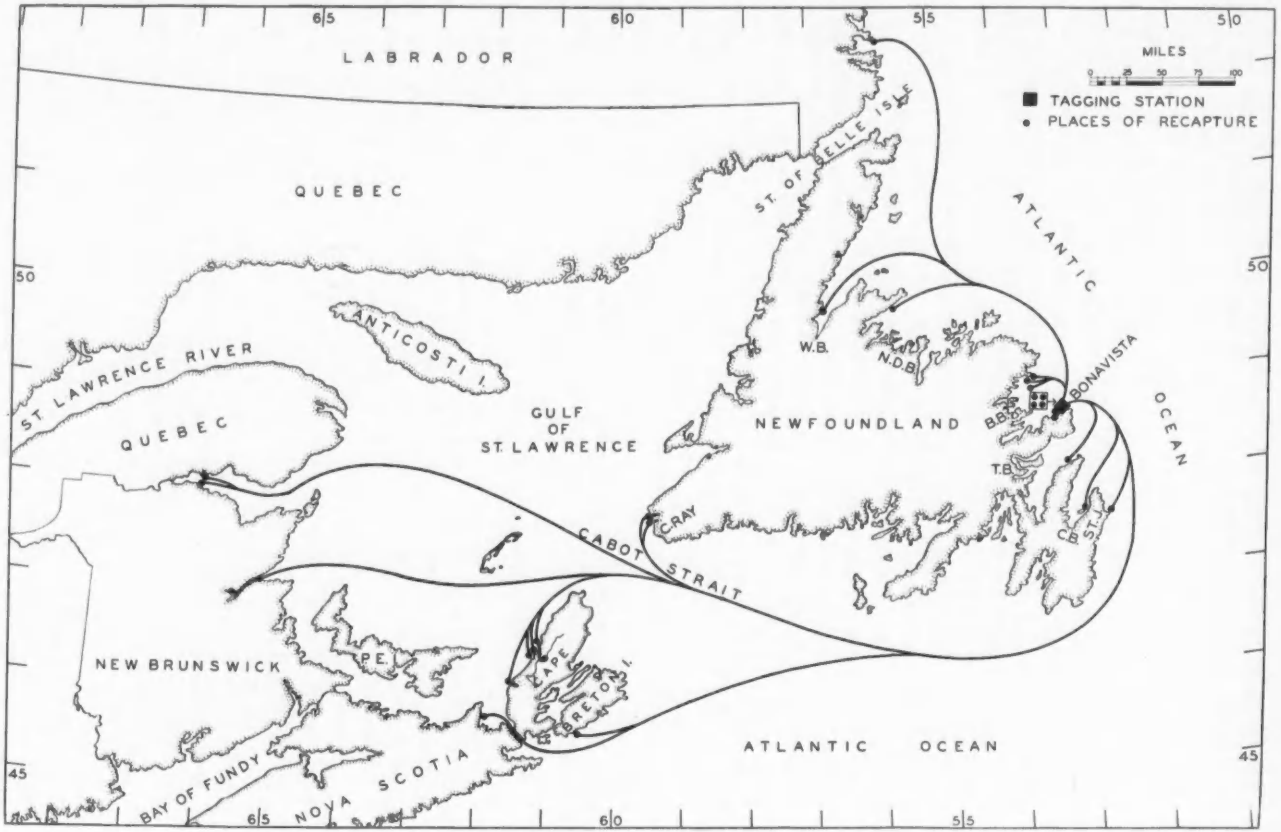

FIG. 2. Distribution of salmon recaptured in 1940, the year of tagging.

in Newfoundland and 60% on the mainland. Thus it is apparent from the results of tagging at Bonavista and St. Anthony on the east coast and at Port aux Basques on the south coast of Newfoundland that a good many of the salmon taken in Newfoundland waters are headed for mainland rivers. In 1951 a large number of smolts on two New Brunswick rivers, Miramichi and Pollett, were marked by fin-clipping (Kerswill, 1955). Of special interest here is the fact that subsequent search has revealed that many of these marked salmon were caught by commercial fishermen on the east coast of Newfoundland.

The two main areas where salmon tagged at Bonavista were recaptured in 1940 were the east coast of Newfoundland (50.0%) and Nova Scotia (25.0%). Since the salmon were tagged on the east coast of Newfoundland, a large percentage would be expected to be recaptured there. Of the 14 salmon recaptured on the east coast, 9 were taken in Bonavista Bay. Four of these are not shown in Fig. 2, three being taken at the tagging station and one 4½ miles from there. Six of the 7 recaptures reported from Nova Scotia were taken on Cape Breton Island, 5 being within 30 miles of the Margaree River.

One of these recaptures is interesting since the adipose fin was missing, and apparently it was one of 31,359 Atlantic salmon smolts so marked by Huntsman (1942) in the Northeast Margaree River about 2 miles above Margaree Forks in 1938. It was recaptured June 17, 1940, at Bonavista in a salmon trap operated by Mr. A. Dyke, and was then tagged and liberated. The scales taken from this fish when tagged at Bonavista showed 2+ years of sea growth, thus agreeing with the interval between smolt marking and recapture. It was recaptured a second time by Mr. M. R. Jackson, an angler, on Sept. 21, 1940, in Upper McDaniel Pool on the Northeast Margaree River about 4 miles above Margaree Forks and about 12 miles from the mouth of the estuary. It was then reported to be 28½ inches (72.4 cm.) long weighing 7½ pounds (3.4 kg.). The distance between Bonavista and Upper McDaniel Pool is 570 miles by the shortest water route and the interval between tagging and recapture was 96 days, a rate of 5.9 miles per day. This rate is low for salmon travelling that distance; possibly the fish spent some time in Margaree River before being recaptured. Concerning migration and homing of salmon Huntsman (1937) says: "On inquiry and examination of the literature I have failed to find a single clear case of a salmon returning to its natal river from a distant place in the sea, that is, away from the neighborhood of the river mouth. Admittedly this is a difficult thing to prove, since we must be sure of three things for the individual fish: (1) Which is its natal river? (2) where it has been in the sea, and (3) that it is again in its river". The double recapture here reported is one instance where these requirements are fulfilled provided, of course, it can be accepted that lack of an adipose fin is sufficient to establish identity. Aside from the question of identity it is believed that more instances of distant recaptures would be reported from marking smolts by attaching tags with directions thereon than from marking by removal of fins, if a suitable tag could be found. In the case of the double recapture referred to above neither the fisherman at Bonavista nor the angler on the Margaree River reported that the fish lacked an adipose fin.

FIG. 2. Distribution of salmon recaptured in 1940, the year of tagging.

Only two (6.9%) of the 29 recaptured salmon were taken by angling. Both of these are referred to above. One was taken in 1940 in the Northeast Margaree River, N.S., representing 3.6% of the recaptured salmon taken in 1940. The other was a kelt taken in 1941 on the Northwest Miramichi River, N.B., being the only recapture in that year. None was taken by angling in Newfoundland rivers. All the other recaptured salmon were caught by salmon shore nets or salmon traps with the exception of one which is peculiar in that it was caught over the side of a boat with a "hook", presumably a boat hook or a gaff, as it was swimming by near the surface of the water. It was reported to be going very fast when captured, but it is incredible that a normal salmon could be caught in such a manner. It was taken 32 miles from the tagging station 6 days after being tagged.

Sea Age

A sample of scales was taken from each fish when tagged, and the sea age, i.e. number of years spent in the sea, was determined later by examination of the scales under a projector. Of the 68 tagged salmon 73.5% spent 2 years in the sea, 13.2% 3 years, 2.9% 4 years, and 10.3% had previously spawned once or twice (Table III). All the scales in the sample from one fish were regenerated

Table III.—Sea age of salmon tagged at Bonavista in 1940 and of various samples taken in 1939, expressed as percentage of the sample.

Place	Date	Year	Sea age				Number of fish
			2 years	3 years	4 years	Previously spawned	
			%	%	%	%	
Bonavista (tagged)	June 8–28	1940	73.5	13.2	2.9	10.3	68
Bonavista	June 26–27	1939	86.8	7.5	...	5.7	53
Bonavista Bay	June 16–July 6	1939	88.1	2.3	0.3	9.3	344
East coast Newfoundland and Labrador	June 2–July 21	1939	79.8	3.7	0.04	16.5	2,544

so that the age could not be determined with any certainty, and this fish is included with those spending 2 years in the sea. Of the previously spawned fish 6 had spawned once and one had spawned twice.

The salmon tagged at Bonavista in 1940 agree with the salmon sampled in 1939 at Bonavista, in Bonavista Bay, and along the east coast of Newfoundland and Labrador (Blair, 1943) in the preponderance of fish spending 2 years in the sea (Table III). They differ, however, in the much higher proportion of fish spending 3 and 4 years in the sea. In 1939 the percentages of fish spending 3 years in the sea in sections 1 to 9 (south to north) of the east coast of Newfoundland and Labrador were respectively: 5.8, 5.7, 9.6, 2.3 (Bonavista Bay), 4.8 (Notre Dame Bay), 1.0, 0.6, 2.4, and 2.1 (Blair, 1943). In sections to the south of Bonavista Bay the proportion was considerably higher than in sections to the north with the exception of the section (Notre Dame Bay) immediately north of Bonavista Bay where it was also relatively high. Although the percentage

of 13.2 for 3-sea-year fish in the salmon tagged at Bonavista in 1940 is somewhat higher than any of the above, some variation is to be expected from year to year on the different parts of the coast.

As regards the recaptured salmon, 2-sea-year fish were taken in practically every locality listed in Table II. Of the 3-sea-year fish one was taken in Conception Bay, which is south of the tagging station, and two were taken in Cape Breton Island (Nova Scotia), the distribution of these recaptures therefore agreeing with the relatively low proportion of fish in this sea class north of Notre Dame Bay in 1939. Only two 4-sea-year fish were tagged and both were recaptured, one in Bonavista Bay and the other on the mainland of Nova Scotia. Two previously-spawned fish were recaptured, one in Bonavista Bay and the other in New Brunswick.

Movements of Recaptured Salmon

The distance between the tagging station and the place of recapture, the interval between tagging and recapture, and the apparent rate of travel for the salmon recaptured in various areas are given in Table IV. Seven recaptured salmon are not included in this table. Three of these were taken at the tagging station, 2 were caught by angling (one in 1940 and the other in 1941), and 2 had insufficient data. The distance between the tagging station and the place of recapture was measured to the nearest quarter mile by the shortest water route. Since the course and actual time taken by the fish cannot be determined, the results should only be considered as approximations to the true rate of movement.

The average distance travelled by the salmon recaptured in Newfoundland was 127.3 miles and by those taken on the mainland 642.5 miles. A salmon taken

Table IV.—Distance between tagging station and place of recapture in miles, interval between tagging and recapture in days, and rate of travel in miles per day for the salmon recaptured in various areas.

Area	No.	Average distance (miles)	Interval in days			Rate in miles per day		
			Av.	Min.	Max.	Av.	Min.	Max
Newfoundland								
Labrador	1	300.0	40.0	...	...	7.5	...	...
White Bay	1	216.75	13.0	...	...	16.7	...	...
Notre Dame Bay	1	156.75	9.0	...	...	17.4	...	...
Bonavista Bay	6	20.0	5.3	3	9	3.7	1.1	11.9
Trinity Bay	1	46.5	6.0	...	...	7.8	...	...
Conception Bay	1	76.5	21.0	...	...	3.6	...	...
St. John's	1	87.5	7.0	...	...	12.5	...	...
Near Cape Ray	2	475.9	28.5	28	29	16.7	16.4	17.0
Nova Scotia	5	569.6	46.8	35	57	12.2	9.7	16.8
New Brunswick	2	754.8	57.0	30	84	13.2	8.6	26.2
Quebec	1	782.75	33.0	...	...	23.7	...	...
Total	22	322.5	25.7	3	84	12.5	1.1	26.2
Summary								
Newfoundland–Labrador	1	300.0	40.0	...	...	7.5	...	...
East coast Newfoundland	11	64.0	8.0	3	21	8.0	1.1	17.4
West coast Newfoundland	2	475.9	28.5	28	29	16.7	16.4	17.0
Other provinces	8	642.5	47.6	30	84	13.5	8.6	26.2

at Upper Charlo, New Brunswick, travelled the greatest distance, namely 785 miles. A close second taken at Miguasha West, Quebec, travelled 783 miles. The average time between tagging and recapture was 11.2 days for salmon taken in Newfoundland and 47.6 days for salmon taken on the mainland. The average rate of travel was 11.4 miles per day for the salmon recaptured in Newfoundland and 13.5 miles per day for those taken on the mainland. The two fastest rates were 26.2 and 23.7 miles per day which were the respective rates for the two salmon reported above as travelling the greatest distances. It will be noticed in Table IV that there is a tendency for the rate of travel to increase with the distance travelled. Using the standard formula for grouped data as given by Davenport and Ekas (1936) the correlation coefficient was found to be +0.687 which is significant since a value that high would not be found in an uncorrelated population as often as once in 100 trials. Similar results were obtained by Menzies (1937, 1938) for salmon tagged on the northwest coast of Scotland in 1936 and on the west coast of Sutherland in 1937, and by Belding and Préfontaine (1938) for salmon tagged at Port aux Basques (Newfoundland) in 1937.

TAGGED GRILSE

Proportion Recaptured

The approximate time of the grilse run in the Bonavista area is between June 10 and July 15 with the peak around June 30. Grilse from the first part of the run were tagged in 1940, the tagging period being June 10 to June 28. Of 386 tagged grilse, 151 (39.1%) were recaptured, 140 (36.3%) being taken in 1940, 10 in 1941, and 1 in 1942 (Table V). All the tagged grilse recaptured in 1940 were

Table V.—Recaptures in 1940, 1941, and 1942 from 386 grilse tagged in 1940.

Year of recapture	1940	1941	1942	Total
Number of recaptures	140	10	1	151
Percentage of total recaptures	92.7	6.6	0.7	100
Percentage of number tagged	36.3	2.6	0.3	39.1

taken between June 10 and Sept. 10, the monthly distribution being: 55 in June, 72 in July, 3 in August, 2 in September, and 8 with indefinite dates. The percentage of the grilse captured in 1940 is slightly less than the percentage of the salmon captured that year, namely 36.3 for the grilse as compared with 39.7 for the salmon. The difference, however, is not so great as would be expected since fishing regulations are designed to favor the capture of salmon. Probably there would have been a greater difference if the salmon had been tagged from the first of their run, as the grilse were.

The 10 recaptures in 1941, the first year after tagging, were taken as follows: 1 in February, 1 in May, 5 in June, 2 in July, and 1 in August. Most of these were probably "kelts" or "mending kelts"—fish which had spawned the previous year. The one recapture in 1942, the second year after tagging, was taken in June. These 11 recaptures taken after the year of tagging represent only 2.8%

of the total number (386) of tagged grilse or 4.5% of the number (246) of tagged grilse not recaptured before spawning.

Since the percentage of grilse which enter the fishery after spawning is so small, it is not economical to restrict the capture of grilse on their first return to fresh water in order to augment the fishery in later years. The grilse could probably be utilized to a greater extent than they are at present. Separate statistics of yearly catches of grilse and salmon should be collected in such a manner that the total catch and the catch per unit effort can be calculated for various areas. In 1939 and 1940 an attempt was made to get such information through the Newfoundland salmon exporters but this method was found to be unsatisfactory and was discontinued. The best alternative method would seem to be to license the fishermen and get the required information from them.

Distribution of Recaptures

The distribution of grilse recaptured in 1940, 1941, and 1942 is given in Table VI and the distribution of recaptures in 1940 only is shown in Fig. 3. Of the 140 grilse recaptured in 1940, 130 (92.9%) were caught in Newfoundland and 10 (7.1%) on the mainland. Compared with salmon the grilse recaptures show a much greater concentration in Newfoundland waters, which is readily explained by the preponderance of grilse in practically all of the Newfoundland rivers. Most (77.1%) of the grilse recaptured in 1940 were taken on the east

Table VI.—Distribution of recaptured grilse tagged at Bonavista in 1940, in actual numbers and as percentage of the total recaptures.

Area	Year of recapture					
	1940		1941	1942	Total	
	No.	%	No.	No.	No.	%
Newfoundland						
White Bay	4	2.9	2	...	6	4.0
Notre Dame Bay	12	8.6	...	...	12	7.9
Bonavista Bay	60	42.9	...	...	60	39.7
Trinity Bay	23	16.4	1	...	24	15.9
Conception Bay	4	2.9	2	...	6	4.0
St. John's	5	3.6	...	...	5	3.3
Trepassey Bay	2	1.4	...	...	2	1.3
St. Mary's Bay	9	6.4	...	...	9	6.0
Placentia Bay	2	1.4	2	1	5	3.3
Fortune Bay	6	4.3	...	...	6	4.0
S.W. coast	2	1.4	1	...	3	2.0
Bonne Bay	...	...	1	...	1	0.7
St. George's Bay	1	0.7	...	...	1	0.7
Nova Scotia	3	2.1	...	...	3	2.0
New Brunswick	6	4.3	1	...	7	4.6
Quebec	1	0.7	...	...	1	0.7
Total	140	100	10	1	151	100
Summary						
East coast Newfoundland	108	77.1	5	...	113	74.8
South coast Newfoundland	21	15.0	3	1	25	16.6
West coast Newfoundland	1	0.7	1	...	2	1.3
Other provinces	10	7.1	1	...	11	7.3

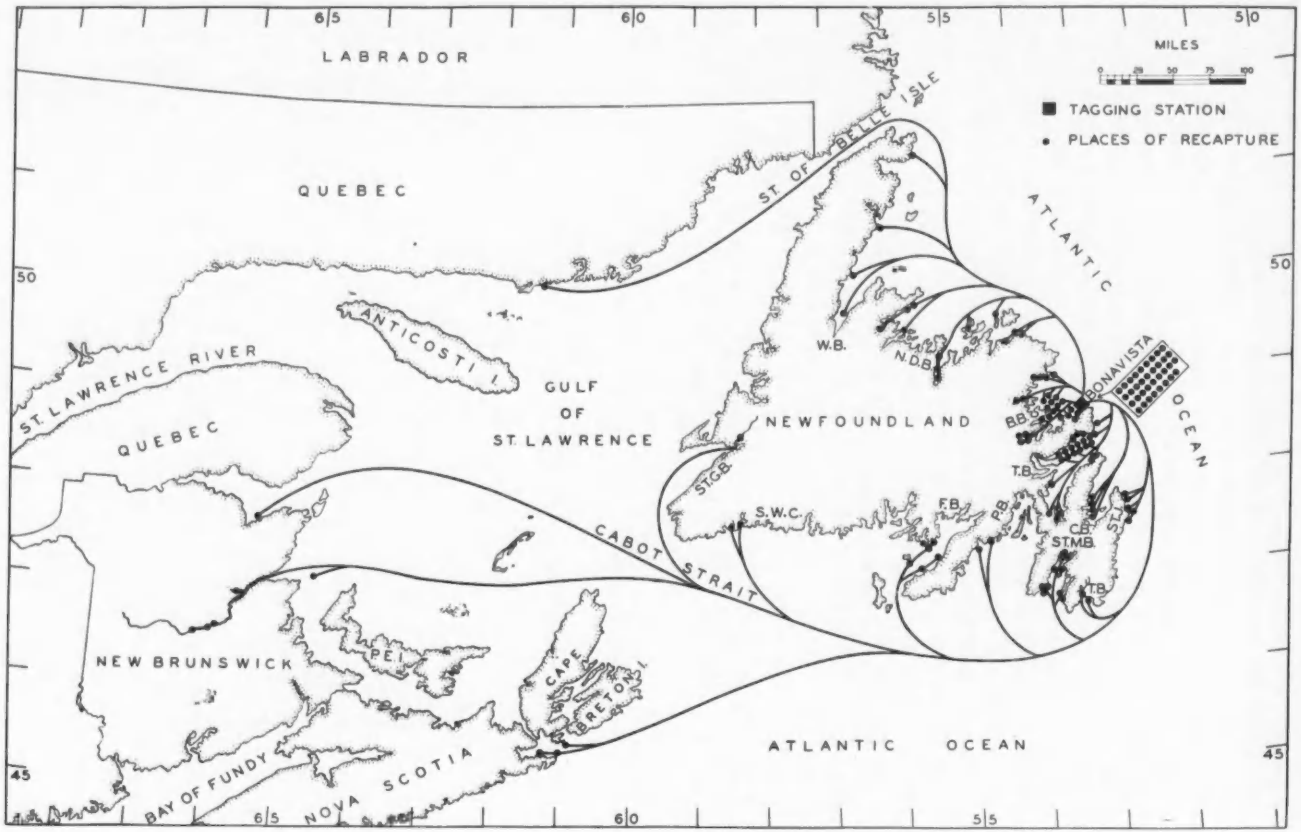

FIG. 3. Distribution of grilse recaptured in 1940, the year of tagging.

coast of Newfoundland but a fair number (15.0%) were taken on the south coast, these two areas accounting for 92.1% of the recaptures.

As for individual areas the majority of the recaptures were from Bonavista Bay where the tagging station was situated. The next two areas in order of importance were Trinity Bay and Notre Dame Bay, adjoining Bonavista Bay on the south and north respectively. Of the 60 grilse recaptured in Bonavista Bay 35 were taken at or within 1½ miles of the tagging station at Bonavista. The distribution of the 11 fish recaptured after 1940 was very similar to the distribution of those taken in 1940.

Only nine (6.0%) of the total number of recaptured grilse were caught by angling. The numbers taken by other means were: 132 by salmon shore nets or salmon traps, 7 by cod traps, 1 by drift net, 1 by trout net, and 1 found dead. The latter was found on the shore of the Southwest Miramichi River, New Brunswick, at Carroll's Crossing and it was reported that "a fish hawk had eaten all of it but the head and a little along its back". Of the nine grilse recaptured by angling 7 were taken in 1940 and 2 in 1941 or, in other words, 5.0% of the grilse recaptured in 1940 and 20.0% of the grilse recaptured in 1941 were taken by angling.

Movements of Recaptured Grilse

The distance between the tagging station and the place of recapture, the interval between tagging and recapture, and the rate of travel for the grilse recaptured in various areas are given in Table VII. The recaptures not included in this table are: 35 taken within 1½ miles of the tagging station, 18 taken after the year of tagging or by angling, and 7 with incomplete data.

Table VII.—Distance between tagging station and place of recapture in miles, interval between tagging and recapture in days, and rate of travel in miles per day for the grilse recaptured in various areas.

Area	No.	Average distance (miles)	Interval in days			Rate in miles per day		
			Av.	Min.	Max.	Av.	Min.	Max.
Newfoundland								
White Bay	4	211.4	25.5	18	41	8.3	5.6	11.4
Notre Dame Bay	11	132.9	16.7	5	57	7.9	2.4	31.6
Bonavista Bay	25	31.7	11.0	2	21	2.9	0.8	8.6
Trinity Bay	21	44.1	14.0	4	46	3.2	0.9	11.6
Conception Bay	3	83.7	7.7	3	14	10.9	6.4	25.8
St. John's	4	84.1	23.0	7	42	3.7	1.8	13.9
St. Mary's Bay	8	205.9	20.8	13	33	9.9	5.7	16.6
Placentia Bay	2	257.5	16.5	11	22	15.6	11.8	23.1
Fortune Bay	6	330.0	20.7	10	29	16.0	11.6	31.9
S.W. coast	1	423.0	21.0	21	21	20.1	20.1	20.1
Nova Scotia	2	549.5	24.0	19	29	22.9	18.7	29.3
New Brunswick	3	726.6	31.0	23	41	23.4	18.2	32.5
Quebec	1	514.75	32.0	32	32	16.1	16.1	16.1
Total	91	142.6	16.3	2	57	8.7	0.8	32.5
Summary								
East coast Newfoundland	68	67.8	14.3	2	57	4.8	0.8	31.6
South coast Newfoundland	17	268.5	20.2	10	33	13.3	5.7	31.9
Other provinces	6	632.2	28.8	19	41	21.9	16.1	32.5

Fig. 3. Distribution of grilse recaptured in 1940, the year of tagging.

The average distance travelled by the grilse recaptured in Newfoundland waters was 108.0 miles and by the grilse taken in mainland waters 632.2 miles. The grilse recaptured on the east coast of Newfoundland travelled an average distance of 67.8 miles and those taken on the south coast 268.5 miles. The greatest distance, 792 miles, was travelled by the grilse which was found dead at Carroll's Crossing on the Southwest Miramichi River, New Brunswick. This record was almost equalled by a grilse taken in the spring of 1941 at Doaktown on the same river, a distance of 787 miles from the tagging station. These distances barely exceed the record distance of 785 miles for salmon tagged at Bonavista.

The average interval between tagging and recapture for grilse taken during the same year on the east coast of Newfoundland was 14.3 days, on the south coast 20.2 days, and on the mainland 28.8 days. The average apparent rate of travel for grilse taken on the east coast of Newfoundland was 4.8 miles per day, on the south coast 13.3 miles per day, and on the mainland 21.9 miles per day. The three fastest rates for individual fish were: 32.5 (near Bathurst, New Brunswick), 31.9 (Brunette, Fortune Bay, Newfoundland), and 31.6 (Lushes Bight, Notre Dame Bay, Newfoundland) miles per day. The record rate for salmon tagged at Bonavista was only 26.2 miles per day. As with salmon, the rate of travel for grilse increased with the distance travelled, the correlation coefficient in this instance being +0.735, which is significant.

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Mr. Harry Brown, manager of the bait depot at Bonavista, and Mr. Max Little, assistant manager, were very helpful in many ways; provision of office space at the depot greatly facilitated business transactions with the fishermen. Mr. Philip Brown of Bonavista helped in the actual tagging operations. Mr. J. H. Molloy, a student technician, assisted in the preparation of data.

REFERENCES

Belding, David L. 1940. Migration of the Atlantic salmon (*Salmo salar*) in the Gulf of St. Lawrence as determined by tagging experiments. *Trans. American Fish. Soc.* for 1939, **69**: 290–295.

Belding, David L., and Georges Préfontaine. 1938. Report on the salmon of the 1937 Port aux Basques (Newfoundland) drift-net fishery. *Contr. Inst. Zool. Univ. Montréal*, No. 3, 58 pp.

Blair, A. A. 1943. Atlantic salmon of the east coast of Newfoundland and Labrador, 1939. *Res. Bull. (Fish.), Dept. Nat. Res., Newfoundland*, No. 13, 21 pp.

Davenport, C. B., and Merle P. Ekas. 1936. Statistical methods in biology, medicine and psychology. John Wiley & Sons, Inc., New York, 216 pp.

Huntsman, A. G. 1937. "Migration" and "homing" of salmon. *Science*, **85**: 313–314.

1942. Return of a marked salmon from a distant place. *Science*, **95**: 381–382.

Kerswill, C. J. 1955. Recent developments in Atlantic salmon research. *Atlantic Salmon J.*, No. 1, pp. 26–30.

Menzies, W. J. M. 1937. The movements of salmon marked in the sea. I. The north-west coast of Scotland in 1936. *Fish. Bd. Scotland, Salmon Fish.*, 1937, No. 1, 17 pp.

1938. The movements of salmon marked in the sea. II. The west coast of Sutherland in 1937. *Ibid.*, 1938, No. 1, 9 pp.

On a Collection of Polychaetous Annelids from Northern Banks Island, from the South Beaufort Sea, and from Northwest Alaska; Together with some New Records from the East Coast of Canada[1]

By E. Berkeley and C. Berkeley
Volunteer Investigators
Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

ABSTRACT

Thirty-seven species of Polychaeta from the western Canadian arctic and northwest Alaska, 27 of which are new to these regions, are enumerated and discussed. Comparison is made with their records in the American arctic to the east of these regions, in the European arctic, and on the east and west coasts of North America. Six species new to the east coast of Canada are also recorded.

INTRODUCTION

The majority of the polychaetes recorded in this paper were collected by Dr. Ferris Neave off the coast of the northern half of Banks Island and off the northwest coast of Alaska when acting as biological observer to the Canadian–United States Beaufort Sea Expedition in the summer of 1954. Dr. Neave's collections were made from the U.S.C.G.C. *Northwind* by means of the "orange-peel" grab and the beam-trawl.

In addition to these records the paper includes others of species collected by members of the scientific personnel of the C.G.M.V. *Cancolim* during the cruises of that vessel in the south Beaufort Sea in 1951 and 1952.

Practically all of the records are of species well known in northern Europe, Greenland, and the European arctic and several of them have been noted previously from Canadian arctic waters either by Chamberlin (1920), by ourselves (1942, 1943, 1944), or by Grainger (1954). However, several are new to the western Canadian arctic and the majority are from a region farther north in the American arctic than where previous records have been made. The nearest geographically, and the most recent, records are those from the Point Barrow, Alaska, region, made by M. Pettibone (1954), whose work has been of great value to us.

We are, further, taking the opportunity to include records of a few species new to eastern Canada collected by Mr. W. L. Klawe at St. Andrews, N. B., and Wedgeport, N. S., in the summer of 1954.

All the arctic records are from Dr. Neave's collection unless it is otherwise indicated. The specimens collected by the *Cancolim* in 1952 were all from one station in the south Beaufort Sea, 69° 35′ N., 136° 10′ W., in 55 metres.

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POLYNOIDAE

Antinoe sarsi Malmgren

Pettibone, 1954, p. 215.

A single specimen from Knight Harbour, Banks Island, in 35 metres. Recorded previously from northern Alaska (Chamberlin, 1920; Pettibone, 1954) and from the western Canadian arctic (Chamberlin, 1920). Not known south of Alaska on the west coast of North America, but from Labrador to Maine (Verrill, 1873) on the east coast. A widespread arctic form.

Eunoe nodosa (Sars)

Pettibone, 1954, p. 217.

A single example in fragments from Prince of Wales Strait, in 50 metres. A widely spread arctic form. Known from northern Alaska (E. and C. Berkeley, 1942; Pettibone, 1954). Not recorded previously from the western Canadian arctic. Until recently known on the west coast of North America south of Alaska by only a single example from Oregon (Hartman and Reish, 1950), but, whilst this paper was in preparation, another one was identified in a collection made by Mr. G. V. Dubokovic, of the Pacific Biological Station, in Alice Arm, B. C., in 104-114 metres. Recorded from the Hudson Bay region (E. and C. Berkeley, 1943; Grainger, 1954) and on the east coast of North America as far south as Massachusetts (Webster and Benedict, 1884).

Eunoe oerstedi Malmgren

Pettibone, 1954, p. 219.

A single example from Prince of Wales Strait, in 55 metres. A widely distributed species, but not recorded previously from the western Canadian arctic. Known from arctic Alaska (Pettibone, 1954), from British Columbia (E. and C. Berkeley, 1948, as *E. barbata*), and as far south as California on the west coast of North America (Hartman, 1939; Moore, 1910, as *E. barbata*); from Labrador (Pettibone, 1954) to Massachusetts (Verrill, 1881) on the east coast.

This species and the foregoing are not easily differentiated and have been frequently confused. Separation here is based as suggested by Pettibone (1954, p. 216).

Harmothoe imbricata (Linné)

Pettibone, 1954, p. 220.

A number of specimens from the northeast extremity of Parry Peninsula in 2.4 to 5.5 metres (*Cancolim*, 1951). A widespread and cosmopolitan species extensively reported from both eastern and western Canadian arctic and along both coasts of North America.

Melaenis loveni Malmgren

Pettibone, 1954, p. 214.

A single fine example, 70 mm. long (*Cancolim*, 1952). The species has not been recorded previously from the western Canadian arctic. Known from northern Alaska, from Bering Sea, and Labrador (Pettibone, 1954), but not farther south on either coast of North America. A widespread European arctic form.

PHYLLODOCIDAE

Phyllodoce (*Anaitides*) *groenlandica* Oersted

Fauvel, 1923, p. 153.

A widespread arctic species. Specimens in the present collection from Cape Prince Alfred, in 35 metres; Prince of Wales Strait in 23 and 55 metres; west of Norway Island in 30 metres; Barter Island, northern Alaska in 20 metres. Also from south Beaufort Sea (*Cancolim*, 1952). Recorded previously from Point Barrow, northern Alaska (Pettibone, 1954), from the western Canadian arctic (Chamberlin, 1920) and from east and south Alaska (E. and C. Berkeley, 1942; Hartman, 1948). South of Alaska it is known from British Columbia (E. and C. Berkeley, 1948), but, apparently, not farther south. Recorded on the east coast of North America from Labrador to Massachusetts (Webster and Benedict, 1884).

Eteone flava (Fabricius)

Fauvel, 1923, p. 173.

A single specimen from west of Norway Island in 30 metres. The only previous records from the Canadian arctic are from Frobisher Bay and Cumberland Sound (Grainger, 1954). The species is recorded from northern Alaska (Pettibone, 1954) and is widespread in the European arctic and northern Europe. Not known from the west coast of North America, but recorded on the east coast from the Bay of Fundy (Treadwell, 1948, as *E. sarsii*).

NEPHTHYDIDAE

Nephthys (*Aglaophamus*) *malmgreni* Théel

Fauvel, 1923, p. 371.

A single specimen from Prince of Wales Strait in 50 metres. A European form with distribution extending from the arctic to the Mediterranean. Previously recorded from Dease Strait, western Canadian arctic (E. and C. Berkeley, 1944), but not elsewhere in the American arctic. On the west coast of North America known only from Behm Canal, southern Alaska (Moore, 1908). Berkeley's (1924) record from British Columbia has been shown to be erroneous (E. and C. Berkeley, 1945), the specimens agreeing with *N. rubella* Michaelsen. Treadwell's record from southern California (1914) is now referred to *Aglaophamus erectans* (Hartman, 1950). The species appears in Whiteaves' "List of Marine Invertebrate of Eastern Canada" (1901) (quoted by McIntosh, 1908 and Treadwell, 1948) as "*N. longisetosa* Oersted" and is said to have been taken "off Anticosti". It does not seem to be recorded farther south on the east coast of North America.

Nephthys ciliata (Müller)

Fauvel, 1923, p. 371; Pettibone, 1954, p. 270.

Three specimens from Icy Cape, Alaska, in 15–16 metres. A widely distributed arctic form. Known previously from Dolphin and Union Strait, western Canadian arctic (Chamberlin, 1920). Recorded from Point Barrow, Alaska (Pettibone, 1954) and from Bering Sea southward, on the west coast of North America, to Washington. Known from the Hudson Bay region (E. and C. Berkeley, 1943; Grainger, 1954) and, on the east coast of North America from Labrador to New England.

SYLLIDAE

Autolytus verrilli Marenzeller (stem-form)

Pettibone, 1954, p. 246 (as *A. alexandri*)

Two examples from St. Andrews, N. B., dredged in shallow depths. The stem-form of the species has not been known previously from Canada. Pettibone (1954) records it from arctic Alaska and Hartman (1945) from North Carolina. We recorded the *Polybostrichus* phase from the Gulf of Georgia in 1954 and there are numerous references to the sexual forms, from the Hudson Bay region (Grainger, 1954), and from various points on both coasts of North America. The species is frequently listed as *A. alexandri* Malmgren, which is, undoubtedly, the oldest name. We use the more recent *A. verrilli* Marenzeller for the reasons given by Marenzeller (1892, p. 416) (see Fauvel, 1914, p. 107).

EUNICIDAE

Onuphis conchylega Sars

Fauvel, 1923, p. 415.

Numerous fine examples from Prince of Wales Strait in 50 and 55 metres. Recorded previously from the western Canadian arctic (E. and C. Berkeley, 1944; Chamberlin, 1920) and from the Hudson Bay region (E. and C. Berkeley, 1943; Grainger, 1954). A widely distributed arctic and subarctic species, known as far south as North Carolina (Hartman, 1945) on the east coast, and California (Hartman, 1944, as *Nothria*) on the west coast of North America.

Lumbrinereis fragilis (O. F. Müller)

Fauvel, 1923, p. 430.

Representatives of this species are from Barnard Point, Melville Sound, in 35 metres; Princess Royal Island, Prince of Wales Strait, in 66 metres; Cape Prince Alfred, in 50 metres; west of Norway Island, in 30 metres; Icy Cape, Alaska, in 15 metres. It has not been recorded previously from the western Canadian arctic, but Pettibone (1954) lists it from the Point Barrow region. Not known south of Alaska on the west coast of North America. Numerous records from the eastern Canadian arctic and southward on the east coast to Massachusetts. A widely distributed European species from the arctic to the Mediterranean.

Lumbrinereis zonata (Johnson)

Hartman, 1944, p. 146.

A single specimen from Princess Royal Island in 66 metres and one from the *Cancolim* (1952). The species has not been recorded previously from the American arctic. Hartman (1948) lists it from Sitka, southern Alaska, her record being the farthest north previous to the present one. It is recorded from British Columbia as *L. brevicirra* (E. and C. Berkeley, 1948) and south to lower California (Hartman, 1944), on the west coast of North America.

FLABELLIGERIDAE

(?) *Brada inhabilis* (Rathke)

Pettibone, 1954, p. 292.

A single specimen from Prince of Wales Strait in 50 metres is very contracted and the identification thereby rendered somewhat uncertain. There is no previous record of the species from the western Canadian arctic. Pettibone (1954) lists it from the Point Barrow region. Recorded from the west coast of North America from south Alaska (Hartman, 1948; Treadwell 1914), but not farther south. Known from the Hudson Bay region (E. and C. Berkeley, 1943; Grainger, 1954), and on the east coast from Labrador to Maine.

SPIONIDAE

Spio setosa Verrill

Verrill, 1873, p. 602; Pettibone, 1954, p. 284.

Several typical examples, collected intertidally, from St. Andrews, N.B., and Wedgeport, N.S. The species has not been recorded previously from Canada. Verrill (1873) and Webster and Benedict (1887) report it from Massachusetts.

Polydora commensalis Andrews

Andrews, 1891a, p. 25 and 1891b, p. 291.

Several specimens from old shells of *Nassa obsoleta* inhabited by the hermit-crab *Pagurus longicarpus* collected at Wedgeport, N.S. The species has not been reported previously from eastern Canada, but is known from British Columbia (E. and C. Berkeley, 1936 and 1952). It is further recorded on the west coast of North America from Oregon, California, and as far south as Mexico (Hartman, 1941), but the original record from North Carolina (Andrews, 1891a) seems to be the only published anticipation of the present one on the east coast. The association in which the present specimens were found is the same as that of the original record.

CIRRATULIDAE

Dodecaceria concharum Oersted

Fauvel, 1927, p. 102.

A single specimen from St. Andrews, N.B., dredged in 15 metres. The species is unrecorded from eastern Canada, but known farther south on the east coast of North America (Verrill, 1879; Webster and Benedict, 1887). It is recorded from British Columbia (E. and C. Berkeley, 1952). A well-known European form.

AMMOCHARIDAE

Ammochares fusiformis (Delle Chiaje)

Fauvel, 1927, p. 203.

A single example taken off Princess Royal Island, in 65 metres. A cosmopolitan form, but not recorded previously from the western Canadian arctic. Known from the eastern Canadian arctic (E. and C. Berkeley, 1943; Grainger, 1954) and south to North Carolina (Hartman, 1945) on the east coast of North America, and from Alaska to California on the west coast.

Myriochele heeri Malmgren

Fauvel, 1927, p. 204; E. and C. Berkeley, 1952, p. 41.

A single small example (10 mm. long, 0.3 mm. wide) from Icy Cape, Alaska, in 15 metres and a large number of larger ones (averaging 30 mm. long and 1.5 mm. wide) from the Beaufort Sea (*Cancolim*, 1952). The small example shows the eye-spots we have described, from small specimens, elsewhere (1952), but these are absent in the larger ones. This species has not been recorded previously from as far north in American waters. It is known from British Columbia (E. and C. Berkeley, 1942) and from Oregon (Hartman and Reish, 1950) on the west coast, and from the Gulf of St. Lawrence (McIntosh, 1913) and from Eastport, Maine, (Webster and Benedict, 1887) on the east coast. A widespread north European and European arctic species.

MALDANIDAE

Axiothella catenata (Malmgren)

Arwidsson, 1906, p. 209.

This species is represented by specimens taken off Cape Prince Alfred in 200 metres; west of Norway Island in 30 metres; and off Icy Cape, Alaska, in 15 metres. It has not been recorded previously from the western Canadian arctic. It is not known from Alaska or the west coast of North America. It is recorded from the eastern Canadian arctic (Chamberlin, 1920; E. and C. Berkeley, 1943; Grainger, 1954) and, on the east coast, from the Gulf of St. Lawrence (McIntosh, 1913) and as far south as New England (Verrill, 1874), but Arwidsson (1906, p. 216) doubts these last records. A widespread northern European and European arctic forms.

Praxillella praetermissa (Malmgren)

Arwidsson, 1906, p. 192; Pettibone, 1954, p. 303.

Specimens from off Cape Prince Alfred in about 35 metres; off Norway Island in 30 metres; and off Barter Island and Icy Cape, Alaska, in 20 and 15 metres respectively. Unrecorded previously from the western Canadian arctic, but collected in the Point Barrow region by Pettibone (1954). Not known from the west coast of North America, but extensively recorded on the east coast from Labrador to Massachusetts. Not known from the Hudson Bay region. A widely distributed northern European and European arctic form.

Leiochone polaris (Théel)

Arwidsson, 1906, p. 150.

A single complete example from Icy Cape, Alaska, in 15 metres. The species has not been recorded previously from North America. Its distribution is regarded as exclusively arctic.

Maldane sarsi Malmgren

Arwidsson, 1906, p. 251; Fauvel, 1927, p. 197.

Specimens from Barnard Point, Melville Sound, in 35 metres; Gore Island, Cape Prince Alfred, in 50 metres; west of Norway Island in 30 metres; Knight

Harbour, Barnes Island, in 30 metres. Also *Cancolim*, 1951, Sta. B4 (71° 52′ N., 125° 19′ W.), in 60 fathoms and *Cancolim*, 1952. Recorded previously from the western Canadian arctic (E. and C. Berkeley, 1942) and from the Hudson Bay region (E. and C. Berkeley, 1943; Grainger, 1954). Listed by Pettibone (1954) from Point Barrow, Alaska. Widely distributed on both coasts of North America and in Europe from the arctic to the Mediterranean.

Rhodine gracilior (Tauber)

Arwidsson, 1906, p. 74.

This species is represented by several fragments from Icy Cape, Alaska, in 15 metres. Amongst these there is only one anterior region, but the species is readily identified by the collars with pectinate edge on the posterior segments and the form of the uncini. The tubes, of which several fragments were collected, are also characteristic. The species has not been recorded previously from the American arctic, but Webster and Benedict (1887) list *Rh. loveni* Malmgren from Eastport, Maine, which species appears to belong here (see Arwidsson, 1906, p. 81). Not known from the west coast of North America. A northern European and European arctic species.

Nicomache sp.? (probably *lumbricalis*)

Arwidsson, 1907, p. 86.

A single, incomplete specimen (*Cancolim*, 1952). The lack of the posterior end prevents certainty of specific identification, but the characters of the anterior end agree with *N. lumbricalis* (Fabricius) which is a very widely distributed arctic form (Pettibone, 1954, p. 305).

SCALIBREGMIDAE

Eumenia crassa Oersted

Fauvel, 1927, p. 127.

A single large (about 70 mm. long) specimen from Princess Royal Island in 66 metres and a smaller one from the *Cancolim*, 1952. The species is not recorded previously from the western American arctic. Grainger (1954) lists it from the Hudson Bay region. Not known from the west coast of North America. Recorded from the Gulf of St. Lawrence and Bay of Fundy (Treadwell, 1948, as *Polyphysia*). A well-known European species, extending from the arctic to the Mediterranean.

CHAETOPTERIDAE

(?) *Spiochaetopterus typicus* Sars

Fauvel, 1927, p. 82.

Several tubes containing only two poorly preserved specimens and some fragments from Cape Prince Alfred in 50 metres. Also some empty tubes from the *Cancolim* (1952). The number of anterior segments (9) and the small number of medium segments (5) point to this species. The tubes are also characteristic. It is not known from the American arctic. McIntosh (1923) records it from the Gulf of St. Lawrence. Not known from western North America. A common north European and European arctic form.

AMPHARETIDAE

Ampharete grubei Malmgren

Fauvel, 1927, p. 227; Pettibone, 1954, p. 316 (as *A. acutifrons*).

Three examples from Icy Cape, Alaska in 16 metres. Not recorded previously from the western Canadian arctic. Know from the Hudson Bay region (Grainger, 1954) and, on the east coast of North America, south to Maine (Webster and Benedict, 1887, as *A. cirrata*). On the west coast from Alaska (E. and C. Berkeley, 1942; Hartman, 1948) to southern California (Moore, 1923). A widely distributed European and European arctic species.

Amphicteis gunneri (Sars)

Fauvel 1927, p. 231; Moore, 1905, p. 849 (as *A. glabra*).

A single example from the *Cancolim* (1952). The species has not been recorded previously from the American arctic. Known on the west coast of North America from southern Alaska (Moore, 1905) and from southern California (Moore, 1923; Treadwell, 1914; E. and C. Berkeley, 1941) and, from the east coast, from the St. Lawrence (*fide* McIntosh, 1922) and from New England (Verrill, 1881). A common European and European arctic form.

Amphicteis sundevalli Malmgren

Malmgren, 1865, p. 366.

A large (74 mm. long) example from the *Cancolim's* 1952 cruise and another smaller and fragmentary one from that of 1951 collected in the same immediate locality. These records are new to the western Canadian arctic. The species is listed from the Hudson Bay region by Grainger (1954) who notes a previous record from the eastern Canadian arctic by McIntosh (1879). It appears to be exclusively arctic (Wesenberg-Lund, 1953).

Asabellides oculata (Webster)

Webster, 1886, p. 157 (as *Sabellides*).

A single example from St. Andrews, N.B., intertidal. The species is new to Canada. It is recorded from New Jersey by Webster (1956) as *Sabellides*. The characters of the species as described agree with those of *Asabellides* (Annenkova, 1929) (= *Pseudosabellides*, Berkeley, 1943).

Asabellides sibirica (Wirén)

Pettibone, 1954, p. 318; Annenkova 1929, p. 494 (as *A. orientalis*).

Several specimens from Icy Cape, Alaska, in 16 metres. Pettibone's record is from Point Barrow, Alaska. We differ from her in her regarding this species as synonymous with our *A. lineata* (1943, as *Pseudosabellides*). The chief point of difference lies in the nature of the uncinigerous pinnules in the abdominal region. In *A. sibirica* these are long, project prominently, and the cirrus is represented by only a low rounded process which does not extend beyond the uncinigerous

lobe (see Annenkova, 1929, Fig. 64); in *A. lineata* they are much shorter, project very little and carry long, slender cirri extending well beyond the uncinigerous lobe. The records of the present species are exclusively arctic.

Asabellides lineata (Berkeley)

E. and C. Berkeley, 1943, p. 131 (as *Pseudosabellides*).

Several specimens from the *Cancolim* (1952). All are differentiated from *A. sibirica* in the manner indicated in the foregoing paragraph. The species has been recorded previously from the western Canadian arctic (E. and C. Berkeley, 1944) and from Alaska (E. and C. Berkeley, 1942, as *Sabellides octocirrata*). From the Hudson Bay region (E. and C. Berkeley, 1943; Grainger, 1954) and from British Columbia (E. and C. Berkeley, 1952). The single, imperfect, specimen described by Treadwell (1943) from Alaska as *Neosabellides alaskensis* probably belongs either to the present or to the foregoing species.

Melinna cristata (Sars)

Fauvel, 1927, p. 237.

A single, exceptionally large (70 mm. long) specimen from west of Norway Island in 30 metres. A new record from the western Canadian arctic. Known from the Hudson Bay region (E. and C. Berkeley, 1943; Grainger, 1954) and from the Gulf of St. Lawrence to North Carolina on the east coast of North America. From Alaska to California on the west coast. A widely distributed European and European arctic form.

Lysippe labiata Malmgren

Malmgren, 1865, p. 367.

A single example from Icy Cape, Alaska, in 16 metres. The only previous record from the American arctic is from the Hudson Bay region (Grainger, 1954). Known from southern Alaska (E. and C. Berkeley, 1942) and from British Columbia (E. and C. Berkeley, 1952) on the west coast of North America, and from New England (Verrill, 1881) on the east. Sparsely distributed in European and European arctic waters.

TEREBELLIDAE

Terebellides stroemi Sars

Fauvel, 1927, p. 291; Pettibone, 1954, p. 330.

Single specimens from Icy Cape, Alaska, in 15 metres and from the west of Norway Island in 30 metres. Previously recorded from the western Canadian arctic (E. and C. Berkeley, 1944), from the Alaskan arctic (Pettibone, 1954), and from the Hudson Bay region (Grainger, 1954; E. and C. Berkeley, 1943). Known on the west coast of North America from Bering Sea to Mexico and on the east coast from Labrador to the Gulf of Mexico. Cosmopolitan.

(?) *Polycirrus eximius* (Leidy)

Verrill, 1873, p. 616.

Several examples of a small (10 to 15 mm. long) blood-red *Polycirrus* collected from the shell of a barnacle taken in a scallop dredge at St. Andrews,

N.B., seem to agree with the rather sketchy description of this species given by Verrill (1873). It has not been recorded previously from Canada but is known on the east coast of North America from New England (Webster, 1879; Andrews, 1891a) south to North Carolina (Hartman 1945).

Neoamphitrite groenlandica (Malmgren)

Fauvel, 1927, p. 250 (as *Amphitrite*).

Three specimens from Prince of Wales Strait in 55 metres. No previous record from the western Canadian arctic. Recorded from Point Barrow, Alaska, (Pettibone, 1954). Known from the Hudson Bay region (Grainger, 1954) and, on the east coast of North America, from New England (Verrill, 1881, as *Amphritrite*); from southern Alaska (Hartman, 1948) but not farther south, on the west coast. A northern European and European arctic species.

Leaena abranchiata Malmgren

Malmgren, 1865, p. 385; Pettibone, 1954, p. 325.

Three specimens from the northeast extremity of Parry Peninsula in 2.4 to 5.5 metres (*Cancolim,* 1951) and a single one from Prince of Wales Strait in 25 metres. The species has not been recorded previously from the western Canadian arctic. It is known from Point Barrow, Alaska, (Pettibone, 1954) and from the Hudson Bay region (E. and C. Berkeley, 1943; Grainger, 1954). It is recorded from Labrador (Pettibone, 1954), but not farther south, on the east coast of North America. From southern Alaska (Hartman, 1948), but not farther south, on the west coast. A widely distributed but almost exclusively arctic species.

ARICIIDAE

Haploscoloplos elongata (Johnson)

Johnson, 1901, p. 412 (as *Scoloplos*); Hartman, 1948, p. 30.

A single example from Icy Cape, Alaska, in 15 metres. The species has not been recorded previously from the western Canadian arctic, but some of the specimens listed by Pettibone (1954) from the Point Barrow region, as *Scoloplos armiger,* may belong here. It is known from the Hudson Bay region (Grainger, 1954) but has not been recorded on the east coast of North America. Its distribution on the west coast extends from southern Alaska to California.

We agree with Monro (1936) and Hartman (1948), and differ from Pettibone (1954), in regarding the absence of crotchets or subuluncini in thoracic neuropodia as differentiating the genus *Haposcoloplos* from *Scoloplos.* They are lacking in the present instance. The specimen differs from *Haploscoloplos alaskensis* (Hartman, 1948) in the absence of a subpodal lobe.

PECTINARIIDAE

Pectinaria (*Cistenides*) *granulata* (Linné)

Malmgren, 1865, p. 359; Pettibone, 1954, p. 312.

A single specimen from Icy Cape, Alaska, in 16 metres. The species is already recorded from the western Canadian arctic (Chamberlin, 1920; E. and C.

Berkeley, 1944). Pettibone (1954) records it from arctic Alaska. Known from the Hudson Bay region (E. and C. Berkeley, 1943; Grainger, 1954). Extending on the west coast from the Bering Sea to Mexico (Hartman, 1941) and on the east coast from Labrador to New England. A widely distributed arctic form.

SABELLIDAE

Chone infundibuliformis Kröyer

Fauvel, 1927, p. 234.

A small, incomplete, specimen from Prince of Wales Strait in 55 metres. The species is recorded from the Alaskan arctic by Pettibone (1954) but not from the Canadian western arctic. Known from the Hudson Bay region (E. and C. Berkeley, 1943; Grainger, 1954). Recorded on the west coast of North America from Bering Sea to California and, on the east coast as far south as New England (Verrill, 1885, as *Sabella picta*). A common arctic form.

Euchone papillosa (Sars)

Malmgren, 1865, p. 407.

Several specimens from the *Cancolim* (1952). The species has not been reported previously from the American arctic. It is known from Labrador (Treadwell, 1948, as *E. tuberculosa*) and on the east coast from New England (Verrill, 1881, as *E. tuberculosa*).There is no record from the west coast. A widespread European arctic form.

The species inhabits extremely long, fine, mud tubes and is easily differentiated from *E. analis* (Kröyer) (which is widely recorded from the American arctic) by the relatively small number of branchial filaments, the entire edge of the anal depression, and the nature of the ventral scutes (see Wesenberg-Lund, 1950).

DISTRIBUTION

An attempt has been made in this paper to relate the occurrence of the species enumerated with the previous records of each from (1) the Alaskan arctic, (2) the eastern Canadian arctic, (3) the west coast of North America, (4) the east coast, (5) the northern seas east of the American continent (including Greenland, Iceland, Northern Europe, and the European arctic). The results are summarized in the accompanying table (page 244).

Only the most general conclusions can be drawn from this table. The first and most obvious is that, as already mentioned in the Introduction, practically all the species which are in the collection are known from the northern seas east of the American continent, whereas several are lacking from the other areas considered. The only exceptions are *Lumbrinereis zonata*, a species believed to be peculiar to the west coast of North America but whose synonymy is in considerable doubt; the two species of *Asabellides*, a genus which has not been generally differentiated from *Sabellides* in the older literature; and *Haploscoloplos elongata*, about whose possible records there is also doubt since the genus *Haploscoloplos* has been separated from *Scoloplos* comparatively recently and

Present records from western Canadian arctic and northwest Alaska	Alaskan arctic	Eastern Canadian arctic	West coast of North America	East coast of North America	Northern seas east of American Continent
Antinoe sarsi	+	−	−	+	+
Eunoe nodosa	+	+	+	+	+
Eunoe oerstedi	+	+	+	+	+
Harmothoe imbricata	+	+	+	+	+
Melaenis loveni	+	−	+	+	+
Phyllodoce groenlandica	+	+	+	+	+
Eteone flava	+	+	−	+	+
Nephthys malmgreni	−	−	+	+	+
Nephthys ciliata	+	+	+	+	+
Onuphis conchylega	−	+	+	+	+
Lumbrinereis fragilis	+	+	+	+	+
Lumbrinereis zonata	−	−	+	−	−
Brada inhabilis	+	+	+	+	+
Ammochares fusiformis	−	+	+	+	+
Myriochele heeri	−	−	+	+	+
Axiothella catenata	−	+	−	+ ?	+
Praxillella praetermissa	+	−	−	+	+
Leiochone polaris	−	−	−	−	+
Maldane sarsi	+	+	+	+	+
Rhodine gracilior	−	−	−	+	+
Nicomache lumbricalis	+	+	+	+	+
Eumenia crassa	−	+	−	+	+
Spiochaetopterus typicus	−	−	−	+	+
Ampharete grubei	+	+	+	+	+
Amphicteis gunneri	−	−	+	+	+
Amphicteis sundevalli	−	+	−	−	+
Asabellides sibirica	+	−	−	−	−
Asabellides lineata	+	+	+	−	−
Melinna cristata	−	+	+	+	+
Lysippe labiata	−	+	+	+	+
Terebellides stroemi	+	+	+	+	+
Neoamphitrite groelandica	+	+	+	+	+
Leaena abranchiata	+	+	+	+	+
Haploscoloplos elongata	+	+	+	−	−
Pectinaria granulata	+	+	+	+	+
Chone infundibuliformis	+	+	+	+	+
Euchone papillosa	−	−	−	+	+

the differentiation has often been disregarded. Circumpolar distribution is thus clearly indicated.

The second point of interest is that, out of the 37 species recorded, 15 are absent from the collection described from the more or less adjacent Alaskan arctic area by Pettibone (1954). It seems unlikely that there is actually so great a difference in the polychaete fauna of the two regions as this would suggest. It is more probable that the collections from both are too small for the comparison to have any definite meaning and that, with further collecting, approximation would be closer.

As regards the occurrence of the species dealt with in this paper on the west and east coasts the only conclusion to be drawn is that there are many more of them on the east than on the west coast. This is in accord with the climatic differences on the two coasts and was to have been anticipated. Comparison with the records from the Hudson Bay region shows as many of the species absent from that area as there are from the west coast. This might have been

expected from a consideration of relative latitudes, less so from that of relative climates. However, in all these cases caution is advisable in drawing any conclusions from the results because the intensity of the collecting effort has been, and still is, very unequally distributed in the various areas and the apparent non-occurrence of any particular species in any particular area can be accepted only with constant regard to this factor.

REFERENCES

ANDREWS, E. A. 1891a. Report upon the Annelida Polychaeta of Beaufort, North Carolina. Proc. *U.S. Nat. Mus.*, **14**.

1891b. A commensal Annelid. *Amer. Naturalist*, **25**.

ANNENKOVA, N. P. 1929. Beiträge zur Kenntnis der Polychaeten-Fauna der USSR. *Ann. Mus. Zool. Acad.* (Leningrad), **30**.

ARWIDSSON, I. 1906. Studien über die skandinavischen und arktischen Maldaniden nebst Zusammenstellung der übrigen bisher bekannten Arten dieser Familie. *Zool. Jahrb.*, Suppl., **9**.

BERKELEY, E. 1924. Polychaetous annelids from the Nanaimo district. Pt. 2. *Contr. Canadian Biol.*, N.S., **2**.

BERKELEY, E. and C. 1936. Notes on Polychaeta from the coast of western Canada. 1. Spionidae. *Ann. and Mag. Nat. Hist.*, Ser. 10, **18**.

1941. On a collection of Polychaeta from southern California. *Bull. Southern California Acad. Sci.*, **40**.

1942. North Pacific Polychaeta, chiefly from the west coast of Vancouver Island, Alaska and Bering Sea. *Canadian J. Res.*, **20**.

1943. Regional and oceanographical conditions in Hudson Bay. 11. Polychaeta from Hudson Bay. *J. Fish. Res. Bd. Canada*, 6.

1944. Polychaeta from the western Canadian Arctic region. *Canadian J. Res.*, **22**.

1945. Notes on Polychaeta from the coast of western Canada. 3. Further notes on Syllidae and some observations on other Polychaeta Errantia. *Ann. and Mag. Nat. Hist.*, Ser. 11, **12**.

1948. Polychaeta Errantia. *Fish. Res. Bd. Canada, Canadian Pacific Fauna*, No. 9b(1).

1952. Polychaeta Sedentaria. *Ibid.*, No. 9c(2).

1954. Additions to the Polychaete fauna of Canada, with comments on some older records. *J. Fish. Res. Bd. Canada*, **11**.

CHAMBERLIN, R. V. 1920. Polychaeta. *Rep. Canadian Arctic Exped.*, 1913–1918, No. 9B. Ottawa.

FAUVEL, P. 1914. Annélides polychètes non-pelagiques provenant des Campagnes de l'Hirondelle et de la Princesse-Alice (1885–1910). *Camp. sci. Monaco, Res.*, **46**.

1923. Polychètes Errantes. *Faune de France*, No. 5.

1927. Polychètes Sédentaires. *Ibid.*, No. 16.

GRAINGER, E. H. 1954. Polychaetous annelids of Ungava Bay, Hudson Strait, Frobisher Bay, and Cumberland Sound. *J. Fish. Res. Bd. Canada*, **11**.

HARTMAN, O. 1939. Polychaetous Annelids. Pt. 1. Aphroditidae to Pisionidae. *Allan Hancock Pacific Exped.*, **7**.

1941. Some contributions to the biology and life-history of Spionidae from California. *ibid.*, **7**.

1944. Polychaetous Annelids. Pt. 5. Eunicea. *Ibid.*, **10**.

1945. The marine annelids of North Carolina. *Duke Univ. Mar. Sta. Bull.*, **2**.

1948. The polychaetous annelids of Alaska. *Pacific Sci.*, **2**.

1950. Goniadidae, Glyceridae, and Nephtyidae. *Allan Hancock Pacific Exped.*, **15**.

HARTMAN, O., AND D. J. REISH. 1950. The marine Annelids of Oregon. *Oregon State Monogr., Studies in Zoology*, No. **6**.

JOHNSON, H. P. 1901. The Polychaeta of the Puget Sound region. *Proc. Boston Soc. Nat. Hist.*, **29**.

MALMGREN, A. J. 1865. Nodiska Hafs-Annulater. *Ofv. Kgl. Svenska Vetenskapsakad., Förhandl.*, **5**.

MARENZELLER, E. 1892. Polychaeta of east Spitsbergen. *Zool. Jahrb.*,**6**.

McINTOSH, W. C. 1879. On the annelids of the British North-polar Expedition. *J. Linn. Soc. London*, **14**.

1908. A monograph of the British Annelids. 2. Pt. 1.

1913. On *Myriochele heeri*, etc., dredged in the Gulf of St. Lawrence, Canada, by Dr. Whiteaves. Notes from the Gatty Marine Laboratory, St. Andrews, No. 35, Pt. 3. *Ann. Mag. Nat. Hist.*, ser. 8, **12**.

1922. A monograph of the British Annelids. 4. Pt. 1.

1923. A monograph of the British Annelids. 4. Pt. 2.

MONRO, C. C. A. 1936. Polychaete worms. Pt. 2. *Discovery Reports*, **12**.

MOORE, J. P. 1905. New species of Ampharetidae and Terebellidae from the north Pacific. *Proc. Acad. Nat. Sci. Philadelphia*, **57**.

1908. Some polychaetous annelids of the northern Pacific coast of North America. *Ibid.*, **60**.

1910. The polychaetous annelids dredged by the U.S.S. *Albatross* off the coast of southern California in 1904. Polynoidae, Aphroditidae, and Segalionidae. *Ibid.*, **62**.

1923. The polychaetous annelids dredged by the U.S.S. *Albatross* off the coast of southern California in 1904. Spionidae to Sabellariidae. *Ibid.*, **75**.

PETTIBONE, M. H. 1954. Marine polychaete worms from Point Barrow, Alaska, with additional records from the north Atlantic and north Pacific. *Proc. U.S. Nat. Mus.*, **103**.

TREADWELL, A. L. 1914. Polychaetous annelids of the Pacific coast in the collection of the zoological museum of the University of California. *Univ. California Pub. Zool.*, **13**.

1943. *Neosabellides alaskensis*, a new species of polychaetous annelid from Alaska. *Amer. Mus. Novitat.*, No. 1235.

1948. Polychaeta. *Fish. Res. Bd. Canada, Canadian Atlantic Fauna*, No. 9b.

VERRILL, A. E. 1873. Report upon the invertebrate animals of Vineyard Sound and the adjacent waters, with an account of the physical characters of the region. *U.S. Comm. Fish.*, Rep. for 1871–1872.

1874. Explorations of Casco Bay by the U.S. Fish Commission in 1873. *Proc. Amer. Ass. Adv. Sci.*, **22**.

1879. Notice of recent additions to the marine invertebrata of the northeastern coast of America, with descriptions of new genera and species and critical remarks on others. *Proc. U.S. Nat. Mus.*, **2**.

1881. New England Annelida. Pt. 1. Historical sketch, with annotated list of the species hitherto recorded. *Trans. Connecticut Acad. Sci.*, **4**.

1885. Notice of recent additions to the marine invertebrata of the northeastern coast of America, with descriptions of new genera and species and critical remarks on others. *Proc. U.S. Nat. Mus.*, **8**.

WEBSTER, H. E. 1879. Annelida Chaetopoda of the Virginian coast. *Trans. Albany Inst.* (New York), **9**.

1886. Annelida Chaetopoda of New Jersey. *New York State Mus. Nat. Hist., Ann. Rep.*, No. 39.

WEBSTER, H. E., AND E. B. BENEDICT. 1884. The Annelida Chaetopoda from Provincetown and Wellfleet, Mass. *U.S. Comm. Fish., Rep.* for 1881.

1887. The Annelida Chaetopoda from Eastport, Maine. *U.S. Comm. Fish., Rep.* for 1885.

WESENBERG-LUND, E. 1950. The Polychaeta of west Greenland. *Medd. om Grønland*, **151**.

1953. The Polychaeta of east Greenland. *Ibid.*, **122**.

WHITEAVES, J. F. 1901. Catalogue of the marine invertebrata of eastern Canada. Geol. Surv. Canada. Ottawa.

Effects of Temperature, Salinity and Oxygen on the Survival of the American Lobster[1,2]

By D. W. McLeese
Fisheries Research Board of Canada
Biological Station, St. Andrews, N.B.

CONTENTS

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ABSTRACT

Thermal acclimation for lobsters transferred from 14.5°C. to 23.0°C. is complete in 22 days. Substantial acclimation to low salinity and to low oxygen occurs within one week. Lethal levels of these three factors are not altered by differences in size within the range 16–34 cm., by difference in the areas where lobsters were caught, or by starvation for up to 57 days. Moulting lobsters are less resistant to high temperature, low salinity and low oxygen conditions than hard-shelled lobsters.

Upper lethal temperature levels and lower lethal salinity and oxygen levels were investigated for hard-shelled lobsters acclimated to each of the 27 combinations of three levels of temperature (5, 15 and 25°C.), salinity (20, 25 and 30‰), and oxygen (2.9, 4.3 and 6.4 mg./l.). The upper lethal temperature is raised by an increase in thermal acclimation, and is lowered by a decrease in the salinity and oxygen acclimation levels. The lower lethal salinity is raised by an increase in the level of thermal acclimation and a decrease in the level of oxygen acclimation. It is lowered by acclimation to reduced salinity. The effect of salinity acclimation is not always the same, but depends on the temperature acclimation. The lower lethal oxygen is raised by either an increase in the temperature acclimation level or a decrease in the salinity acclimation.

The lower lethal temperature is 1.8°C. for 17° acclimated lobsters, and 5.0° for 27.5° acclimated lobsters.

Ultimate and maximum or minimum lethal levels of temperature, salinity and oxygen—the highest and lowest lethal levels that can be attained by acclimation—were interpolated from the results. These measures were used to integrate the lethal levels of the three factors into a single three-dimensional graph which describes the boundary of lethal conditions for lobsters exposed to the three factors operating together (Fig. 7).

INTRODUCTION

Wide seasonal and short-term variations in temperature, sporadic variation in salinity, and reduction of dissolved oxygen are known to occur and either singly or in combination may result in conditions that are unsuitable for lobster survival. Heavy mortalities occur at times among lobsters that are held in large numbers by the industry. Sometimes the mortalities have been attributed to disease, but in general, adverse environmental conditions have been suspected.

In this investigation the lethal effects of temperature, salinity and oxygen were studied to determine the actual levels of these three factors that restrict lobster survival both in nature and in commercial holding units.

Fry (1947) provided a basis for understanding the relationships and interactions of various environmental influences on an organism. He stressed the idea that previous experience or acclimation modifies the physiology of an organism and has a pronounced effect on its response to environmental factors. Unless acclimation is considered in relation to the factor being studied, results will not have a precise meaning. His concepts were developed from experimental evidence derived from the study of fish. The present paper illustrates that the same concepts also apply to the invertebrate field.

Physiological changes associated with moulting (Hollett, 1943; Lowndes and Panikkar, 1941; Donahue, 1953) may alter the response of lobsters to their environment. In addition there are physiological differences associated with variations in life-history among lobsters from different areas, (Templeman, 1936a; Wilder, 1953). Separate tests were performed to establish the effects of such physiological differences on lobster survival.

Some preliminary work on the lethal levels of temperature, salinity and oxygen for lobsters appears in the literature. Chaisson (1932) held lobsters successfully for several days in aerated sea water at 25°C. Lobster larvae were reared to the fourth stage at 24°C. by Templeman (1936b).

Wood (1885) tested the survival of lobsters at low temperatures. Three out of five lobsters transferred from water at 21°C. to water at the freezing point were dead by 24 hours.

Chaisson (1931) found that the respiration of 23-cm. lobsters stopped after 7½ hours at a salinity of 11.4‰ and after 13 hours at 19.5‰ when the temperature was between 11° and 13°C. Templeman (1936b) reared lobster larvae to the fourth stage at salinities between 18‰ and 35‰ at temperatures between 15° and 20°C. He concluded that 21‰ was almost as satisfactory for their survival as the normal 31‰. Salinities lower than 19.4‰, however, were definitely unfavourable and at 16.4‰ larvae died before moulting into the third stage.

Some information on low oxygen tolerance was supplied by Chaisson (1932). Four glass jars of 3-quart capacity, each with one 23-cm. lobster, were filled with water saturated with oxygen and sealed. Two of the lobsters were held at 13.5° to 15.0°C. and died after 18 hours. The other two, which were held at 0.5° to 1.0°C., were still living after 60 hours. He concluded that lobsters are most resistant to low oxygen at low temperatures. The final oxygen contents of the water were not recorded and a low rate of oxygen consumption at low temperatures could account for the observed differences.

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MATERIALS

Most of the lobsters were obtained from the southwestern shore of New Brunswick on the Bay of Fundy. Others came from eastern New Brunswick on the Gulf of St. Lawrence, Prince Edward Island and Cape Breton, Nova Scotia. The stocks of lobsters used in the experiments are listed in Table I.

In addition to the lobsters listed in Table I, 280, 250 and 450 lobsters were used during 1949, 1950 and 1951, respectively, in lethal temperature, salinity and oxygen experiments. The results of these preliminary experiments are not reported in this paper.

The lobsters from Prince Edward Island measured between 22 and 26 cm. Those from eastern New Brunswick ranged in size from 16 to 28 cm. total length

TABLE I.—Stocks of lobsters used in the experiments.

Area	Date and number
Prince Edward Island	August, 1949 (120)
Eastern New Brunswick	June, 1950 (200)
Southwestern New Brunswick	June, 1950 (474); June, 1951 (70); May, 1952 (600); June, 1952 (1400); July, 1952 (400); Jan., 1953 (1550); May, 1953 (500); June, 1953 (1150).
	Total: 6464

(tip of rostrum to tip of telson). The lobsters from southwestern New Brunswick ranged in size from 20 to 28 cm., but most ranged from 22 to 26 cm.

The inshore water of the Gulf of St. Lawrence is warmer in summer and colder in winter than the water of the Bay of Fundy. Lobsters from the two areas differ in life-histories (Templeman, 1936a). From his observations, Templeman (1944) estimated that half the female lobsters from the warm areas of the Gulf of St. Lawrence have become mature at a length of 23.5 cm. and half those from southwestern New Brunswick have become mature at 38.0 cm. Judging from the sizes of the lobsters that were used in the experiments, it appears that most of those from the Gulf of St. Lawrence and virtually all those from southwestern New Brunswick were immature.

Some moulting occurs during the late spring and early summer months and a small number of the lobsters obtained for the experiments were preparing to moult. These were segregated and special tests were performed, using the premoult lobsters and those that subsequently moulted. The remaining lobsters were hard shelled and were not preparing to moult. These were used in all the other experiments.

During the period between the time lobsters were obtained and placed in controlled acclimation tanks, they were held in floating crates and a floating car at the end of the wharf at the Biological Station, St. Andrews, N.B. They were fed herring and flounder during these holding periods. They were not fed, however, during controlled acclimation because of the danger of water pollution at high acclimation temperatures and reduced flows. Evidence is presented in a later section to show that lack of food during acclimation had no appreciable effect on the lethal temperature.

METHODS

ACCLIMATION TANKS AND CONTROLS

Three wooden acclimation tanks were used in 1949 and 1950. They measured 3 by 3 feet by 6 inches (91 × 91 × 15 cm.). These were replaced in 1951 by nine wooden tanks measuring 4 feet by 4 feet by 8 inches (122 × 122 × 20 cm.). In addition, three concrete tanks, 7 by 3.5 feet with 6 inches of water (213 × 107 × 15 cm.) were used for acclimation in 1952. Thirty lobsters at a time were held in the smallest tanks and 50 were held in the others.

During 1949 and 1950, temperatures were maintained in the three acclimation tanks by adding small flows of heated sea water from a constant level tank maintained at a constant temperature. Final temperature control in the ac-

climation tanks was obtained by thermostatically controlled pyrex glass immersion heaters in each acclimation tank. Temperature fluctuated less than ±1.0°C.

During 1951, 1952 and 1953, water was heated as it passed through two or three 400-watt, pyrex glass-lined heaters installed over each tank. The final temperature control was obtained by thermostatically controlled pyrex-insulated immersion heaters. Temperature fluctuated less than ±1.0°C.

Reduced salinities were obtained by mixing fresh water with inflowing sea water in the desired proportions. Salinities were determined by hydrometer and reference to salinity tables, Knudsen (1946). Salinity fluctuated less than ±1.0‰.

Compressed air delivered through four or five aquarium-type air stones in each tank kept the oxygen content over 4.3 mg. O_2/l. during 1949 to 1951, inclusive. During 1952 and 1953, 6.4 mg. O_2/l. were maintained during temperature and salinity acclimation. Reduced levels of oxygen were obtained by reducing the flow of air to the tanks. Oxygen was determined by the unmodified Winkler method. The oxygen content fluctuated less than ±1.0 mg. O_2/l.

Lethal Tanks and Controls

Two lethal tanks similar to the smallest acclimation tanks were used in 1949 and 1950. High temperatures were obtained by adding small flows of heated sea water from a constant level tank maintained at a constant temperature. Test temperatures fluctuated less than ±0.3°C.

Two lethal tanks measuring 2 by 2 feet by 6 inches (61 × 61 × 15 cm.) were used in 1951 and 12 were used during 1952 and 1953. They were constructed of unpainted 1-inch (2.5-cm.) pine lumber. The method of heating was the same as that used for the larger acclimation tanks. Temperature was controlled within ±0.4°C. for lethal temperature tests and within ±1.0°C. for lethal salinity and oxygen tests.

Reduced salinities were obtained by mixing fresh water and sea water in constant level buffer tanks. Salinity was controlled within ±0.5‰.

The required oxygen contents for the lethal temperature and salinity tests were obtained by adjusting the amount of compressed air supplied to each tank. Reduced oxygens for the lethal oxygen experiments were provided by apparatus working on the principle described by Fry (1951). A glass cylinder 4 feet (122 cm.) long by 4 inches (10.1 cm.) in diameter was filled with stones of approximately ½-inch (1.3-cm.) diameter. Nitrogen that was bubbled up through the column from beneath displaced oxygen from water entering the column at the top. The oxygen content of the water leaving the column was regulated by adjusting the flows of nitrogen and water until the desired oxygen level was attained. Glass plates placed over the surface of the test tanks reduced oxygen exchange with the air to a minimum. Oxygen was controlled within ±0.3 mg. O_2/l.

Determination of Death

Lobsters were considered dead when no movement of any part could be detected upon close examination. "Dead" lobsters did not recover from temperature or salinity deaths when they were returned to the appropriate acclimation conditions. Thorough inspection of each animal was necessary before death

could be determined in low oxygen experiments since lobsters that are very weak and appear to be dead from the effects of low oxygen can recover completely within 1 to 2 hours when returned to acclimation conditions.

ACCLIMATION

Any study of the lethal effects imposed upon an organism by environmental extremes must be based on a knowledge of its ability to acclimate, for then its environmental history can be stabilized to yield comparable data. Temperature, salinity and oxygen acclimation for the lobster are treated below.

Temperature Acclimation

Acclimation to a new level of temperature was followed using the same method as Loeb and Wasteneys (1912) and Brett (1946). A test temperature at which average survival time is short at the start of acclimation but reaches a maximum when acclimation is complete, was selected on the basis of preliminary tests. At completion of acclimation, the average survival time was taken as 72 hours, the end point of the experiment, if no deaths occurred in the test temperature. As well, in tests where less than 100% mortality occurred, the average survival time was calculated using 72 hours for each of the animals living past that time.

Lobsters that had been held at 14.5° C. were transferred to 23.0°C. for acclimation. After exposure from 1 to 31 days, groups of six to ten lobsters were removed and their average survival time at 30°C. was determined.

One hundred per cent mortality occurred within 8 to 16 hours for all groups acclimated for 10 days or less. There was a slight increase in average survival time during this period. By 24 days, the average survival time had increased to 72 hours and acclimation was considered complete. The gain in average survival time is shown in Fig. 1.

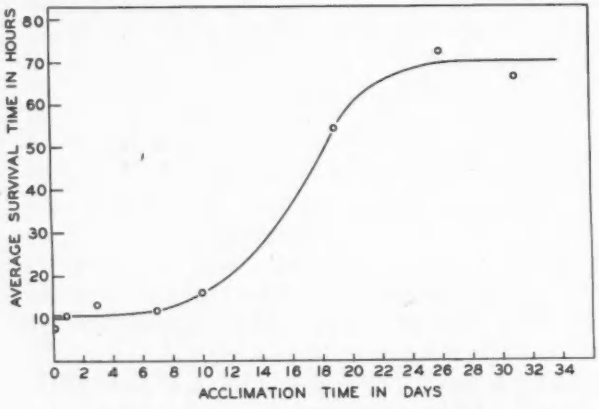

Fig. 1.—Gain in average survival time of lobsters transferred from 14.5°C. to 23.0°C. for 1 to 31 days and then tested at 30°C.

Upward acclimation to high temperature (from 14.5° to 23.0°C.) requires a longer time for lobsters, about 22 days, than for those fish that have been studied. The bullhead (*Ameiurus nebulosus*) acclimates in one day when transferred from 20° to 28°C. (Brett, 1944). The common mummichog (*Fundulus heteroclitus*) requires three days for approximately the same temperature change (Loeb and Wasteneys, 1912). Fast rates of about one day were found for the large mouth black bass (*Huro salmoides*) (Hathaway, 1927), and the marine goby (*Gillichthys mirabilis*) (Sumner and Doudoroff, 1938). The goldfish (*Carassius auratus*) requires about 6 days for acclimation when the temperature is raised from 12° to 20°C. (Brett, 1946).

The differences in acclimation rates are partially a reflection of the latent periods at the beginning of acclimation when there is little change in temperature resistance. The lobster has a 10-day latent period. The goldfish that acclimates in about one quarter the time, has a 17-hour latent period (Brett, 1946). The bullhead has no observed latent period (Brett, 1944).

Salinity Acclimation

As a test for acclimation, lobsters surviving exposure to reduced salinity in preliminary lethal experiments were subjected to an even lower salinity. The survival of these lobsters was compared with the survival of lobsters acclimated in nature to 30‰. The results are presented in Table II.

Table II.—Acclimation of lobsters to reduced salinity.

History	Test conditions	No. of lobsters	Number dead at stated hours								
			1	3	6	9	12	24	48	72	96
From 30‰ at 5° C.	7.5‰ at 5° C.	10	0	0	0	0	0	0	1	3	5
From 15‰ at 5° C. for 96 hours	7.5‰ at 5° C.	10	0	0	0	0	1	1	1	1	2
From 30‰ at 5° C.	3.7‰ at 5° C.	10	0	0	0	0	5	10	–	–	–
From 15‰ at 5° C. for 96 hours then 7.5‰ for 144 hours at 5° C.	3.7‰ at 5° C.	8	0	0	0	0	0	2	5	–	–
From 30‰ at 17° C.	11.4‰ at 17° C.	10	0	0	1	–	8	10	–	–	–
From 18.7‰ at 17° C. for 168 hours	11.4‰ at 17° C.	9	0	0	0	–	0	1	–	–	–
From 15‰ at 17° C. for 168 hours	11.7‰ at 17° C.	8	0	0	0	–	0	0	–	–	–

The results are not strictly comparable, since some mortalities occurred during the initial exposure of the acclimated groups. However, the mortalities were not greater than 20% and the selection was not severe. It is concluded on the basis of the different survival of the groups that substantial acclimation had occurred within four to seven days. Whether or not acclimation was complete in 4 to 7 days cannot be determined from these results, but in later experiments acclimations of 7 to 14 days were used.

A fast adjustment of lobster blood salinity to external salinity is indicated by the work of Smith (1951). He found that within the first 12 hours of exposure to low salinity the lobster blood often became more dilute than the external medium. This was followed by an increase in the internal concentration

until at about 24 hours the two concentrations were nearly equal and further adjustments were usually small. If these changes are related to acclimation, the latter may be substantially complete in the same time.

Other marine animals have been shown to have an increased resistance to low salinities when acclimated to reduced salinities. These are the prawn *Palaemonetes varians* (Panikkar, 1941), the marine worm *Nereis virens* (Topping and Fuller, 1942), and the oyster *Ostrea virginica* (Loosanoff and Smith, 1949). Loosanoff (1945) found no evidence of acclimation to reduced salinity in the starfish *Asterias forbesi.*

Oxygen Acclimation

As a test for acclimation to reduced oxygen, lobsters were exposed to reduced oxygen for four days. Their survival time in low oxygen was compared with the survival times of lobsters that had been held at full oxygen. The results are presented in Table III.

Table III.—Acclimation of lobsters to reduced oxygen.

Acclimation temp.	Acclimation oxygen	Test oxygen	Number dead at stated hours; ten animals in each test.										
			6	9	12	18	24	36	48	60	72	84	96
° C.	*mg./l.*	*mg./l.*											
15–16	3.7	0.8	0	0	0	0	0	0	0	0	0	0	0
15–16	6.4	0.8	0	0	0	0	1	4	4	6	7	8	9
24–25	3.6	1.2	0	0	0	0	3	3	3	3			
24–25	6.4	1.1	0	0	0	0	0	2	6	6			
24–25	2.9	0.6	0	1	1	3	7	10					
24–25	6.4	0.7	0	3	4	7	10	10					

A definite increase in survival time is apparent for lobsters from reduced oxygen compared to the survival times of lobsters from high oxygen when placed in the same low oxygen test conditions. The exact rate of acclimation cannot be determined from these results.

Acclimation to reduced oxygen has been shown for fish. Young salmon (*Salmo salar*) were shown to acclimate to reduced levels of oxygen (Nikiforof, 1953). Changes in the rate of respiration of animals reared in different oxygen conditions were used as an index of acclimation. Shepard (1955) found that acclimation to reduced levels of oxygen in the speckled trout (*Salvelinus fontinalis*) lowered the lethal oxygen level.

PRELIMINARY CONSIDERATIONS FOR LETHAL LEVELS

All the lobsters used in determining the lethal levels of temperature, salinity and oxygen were hard-shelled lobsters of a restricted size range and from one area. This selection was made to rule out any possible relationships between resistance to environmental factors and size, geographic variation, and, the moult cycle. To interpret the scope of results obtained using a restricted stock, it is important to know if such relationships exist. The effects of these items

and the possible effect of starvation were given preliminary consideration. The results are discussed in the following sections.

Size in Relation to Lethal Levels of Temperature, Salinity and Oxygen

TEMPERATURE. There is a diversity of response reported in the literature concerning size in relation to order of death of animals at high temperature. Huntsman and Sparks (1924) and Bélehrádek (1935) claim that resistance to heat diminishes as size increases. Hart (1952) found a size effect in only three of the fourteen species of freshwater fish that he studied. In two of these species, resistance diminished with increasing size but in the third, resistance increased.

Sumner and Doudoroff (1938), Doudoroff (1942) and Fry, Hart and Walker (1946) concluded that there was no correlation between size of individual specimens of the marine goby (*Gillichthys mirabilis*), the greenfish (*Girella nigricans*), and the speckled trout (*Salvelinus fontinalis*) and order of death at high temperature.

Tests were performed with lobsters of two size groups, 21 to 22 cm. and 26 to 28 cm., to determine if their size is related to death at high temperatures. The lobsters were acclimated at 9°C. and tested by placing ten of each size group in constant temperature baths at 25°, 26°, 27° and 29°C.

For both size groups the temperature that killed 50% of the animals in 48 hours was 26.5°C. It was concluded that small (21 to 22 cm.) and large (26 to 28 cm.) lobsters from the same area and acclimated to the same temperature respond identically to high temperature. Within the range tested, size does not appreciably affect the resistance of lobsters to high temperatures.

SALINITY. Bert (1871) concluded that larger fish were more resistant to reduced salinity than smaller fish of the same species. On the other hand, Topping and Fuller (1942) concluded from their study of fourteen species of marine invertebrates that, in general, smaller animals of a particular species were better able to survive in reduced salinities. Quigley (1928) reported no size relationship in the resistance of dogfish (*Squalus suckleyi*) to reduced salinities. Experiments were performed to test the effect of size on the lethal salinity of lobsters. Lobsters measuring 16 to 18 and 21 to 28 cm. were acclimated at 13°C. and others measuring 16 to 23 and 29 to 34 cm. were acclimated at 25°C.

The data are available in Tables IX and X of McLeese (1950). In general, they suggest but are not quite sufficient to prove that small lobsters are more resistant to reduced salinities than large lobsters, over the range 16 to 34 centimetres. At 13°C., the salinity that killed 50% of the lobsters in 48 hours was interpolated as 12.3‰ for the small and 13.2‰ for the large lobsters. At 25°C., the smaller lobsters were more resistant to 15‰ salinity, but less resistant to 19‰ salinity: the interpolated 50% lethal level was 20‰ for the small and 19‰ for the large lobsters. If a size effect is present, it is small in comparison with most of the effects to be tested. In any event, in the main experiments the sizes of lobsters used were restricted to 23 to 26 centimetres, and effects of size within these limits are negligible.

OXYGEN. Size in relation to order of death in low oxygen was not studied. Thomas (1954) working on the European lobster (*Homarus vulgaris*) found that the oxygen uptake of small lobsters is greater per unit weight than that for large lobsters. There is a possibility then that size could be an important factor when lobsters are exposed to low oxygen. Size was eliminated as a variable factor in the lethal oxygen experiments by using lobsters of a limited size range (23 to 26 cm.).

Resistance of Lobsters from Different Areas to Temperature, Salinity and Oxygen

TEMPERATURE. As discussed previously, lobsters from the Gulf of St. Lawrence and those from southwestern New Brunswick differ in their life-histories. Most of the differences are thought to result from different temperature characteristics of the water in these areas. Lobsters from both areas were acclimated at 10°C. and 14.5°C. The temperature that killed 50% of the lobsters in 48 hours was 28°C. for both groups acclimated at 10°C. At 14.5°C. acclimation, lobsters from both areas died in 24 hours when tested at 30°C. The times to death correspond almost exactly. It was concluded that the thermal resistance of both groups is identical when they are acclimated at the same temperature.

Out of ten species of freshwater fish studied by Hart (1952) only three in which sub-species are recognized showed appreciable geographic differences in temperature resistance.

SALINITY AND OXYGEN. The resistance of lobsters from different areas to low salinities and oxygens was not studied. In nature, the various areas where lobsters occur generally differ more in water temperature than they do in salinity and oxygen levels. Since no difference in temperature resistance could be demonstrated for lobsters from different areas, possible differences in salinity and oxygen resistance have been disregarded.

Temperature, Salinity and Oxygen Lethals of Starved and Fed Lobsters

The standard experimental procedure was to starve lobsters for periods up to 45 days during controlled acclimation. There is a possibility that the physiology of animals given this treatment would be modified in some way. For example, Hoar and Dorchester (1949) found that the heat tolerance of goldfish (*Carassius auratus*) was modified when their body fats were altered by feeding the fish special foods.

TEMPERATURE. A lethal temperature experiment using starved and fed lobsters was conducted to determine whether starvation altered the lethal temperature (McLeese, 1954, Table I). Lobsters were acclimated to 15°C. for periods up to 57 days. During this time half the lobsters were fed at the rate of one pound of herring per hundred pounds of lobsters per day. The other half were not fed.

During the acclimation period unexplained mortality reduced the numbers of animals in both groups by about one half. Presumably because of residual debility from this episode, lethal temperatures determined for the survivors were

less than for other similar groups. However the relative performance of the starved and the fed is assumed to be indicative of the normal situation.

Ten starved and ten fed lobsters were tested at each temperature of 26°, 27°, 28° and 29°C. The temperatures that killed half the lobsters in 48 hours were estimated by interpolation to be 27.7° and 27°C. for starved and fed animals, respectively, but the differences were not consistent and were not "significant". I concluded that starvation during acclimation in the experiments below could have little or no effect on the lethal temperature level, especially since the acclimation periods used were on the whole considerably less than 57 days (35 to 49 days).

Brett (1944) studied the effect of starvation on the lethal temperature of the bullhead *Ameiurus nebulosus*. There was no appreciable difference in lethal temperature after 40 days' starvation, even though the weight–length relationships of the fish changed. In tests with lobsters, Wilder (personal communication) found that starvation for periods up to 5 weeks had no significant effect on the weight of lobster muscle.

SALINITY AND OXYGEN. Experiments were started to determine the lethal levels of salinity and oxygen for starved and fed lobsters. Unfortunately, however, unexplained mortality during the acclimation period killed the experimental stock. Considering that the lethal temperature is the same for starved and fed lobsters, it is assumed in this paper that starvation during acclimation does not have an effect on the salinity and oxygen lethals.

Moulting and Resistance to Temperature, Salinity and Oxygen

TEMPERATURE. The moult period has long been recognized as a critical period in the life-history of the lobster because of the susceptibility of soft-shelled lobsters to physical damage. As well, the lobster's physiology undergoes changes at this time. These changes could be extensive enough to produce significant changes in the tolerance of the lobster to environmental extremes.

Five high temperature tests were performed with lobsters shortly after they had moulted. Hard-shelled lobsters were used as controls. The results are presented in Table IV. The average survival time of moulting lobsters is less than that for hard-shelled lobsters.

SALINITY. Four low salinity tests were performed with lobsters shortly before or after they had moulted. Six of the lobsters were in the premoult condition

TABLE IV.—Average time to death at high temperatures of moulting and non-moulting lobsters, acclimated to 15° C. and various levels of salinity and oxygen.

Acclimation level		Test temperature	Number of lobsters	Average time to death	
Salinity	Oxygen			Moulting	Non-moulting
‰	*mg./l.*	° C.		*minutes*	*minutes*
30	4.3	28	4	1022	>1860
30	2.9	28	6	193	>1753
25	6.4	29	3	80	580
25	2.9	27	3	400	640
20	2.9	28	2	135	1080

and two had recently moulted. The results are presented in Table V. The moulting lobsters died in a shorter average time than the control lobsters with the exception of the two premoult lobsters acclimated at 20‰ salinity and 2.9 mg. O_2/l.

TABLE V.—Average time to death in low salinities of moulting and non-moulting lobsters, acclimated at 15° C. and various levels of salinity and oxygen.

Acclimation level		Test salinity	Number of lobsters	Average time to death	
Salinity	Oxygen			Moulting	Non-moulting
‰	*mg./l.*	‰		*minutes*	*minutes*
25	2.9	11	2	360	1980
25	2.9	9	2	480	630
20	2.9	9	2	740	720
30	6.4	13	2	2390	>2880

OXYGEN. A low oxygen experiment was performed with 20 lobsters shortly before or after they had moulted. They were acclimated at 15°C., 30‰ salinity and 6.4 mg. O_2/l. and tested at 0.3 mg. O_2/l. The average survival time of the moulting lobsters was 750 minutes and that of the controls was greater than 1040 minutes.

These tests have shown that moulting lobsters are less resistant than hard-shelled lobsters to high levels of temperature and low levels of salinity and oxygen.

In summary; when acclimation histories are similar, lethal levels of temperature, salinity and oxygen are not influenced by size differences, variation in life-history or short-term starvation. One possible exception is starvation and lethal oxygen levels. The resistance of moulting lobsters to all three factors is less than that for non-moulting lobsters. Consequently, the selection of a restricted stock of lobsters for the experiments has limited the possibility of interpretation of the results to hard-shelled lobsters.

LETHAL LEVELS OF TEMPERATURE, SALINITY AND OXYGEN

Preliminary experiments (McLeese, 1950) showed that lobsters can be acclimated to withstand high temperatures under favourable conditions of salinity and oxygen. Further, the lethal levels of salinity and oxygen vary with the temperature.

To understand the effects of temperature, salinity and oxygen on lobster survival as they operate together, the three factors were varied during acclimation according to a pattern. Lobsters were acclimated to the 27 combinations of three levels of temperature (5, 15, 25°C.) with three levels of salinity (20, 25, 30‰) with three levels of oxygen (2.9, 4.3, 6.4 mg./l.). Enough lobsters were acclimated to each of the 27 combinations to allow for the determination of lethal temperature, lethal salinity and lethal oxygen levels.

All the lobsters acclimated to the 5°C. temperature were caught in the winter months at temperatures of approximately 3 to 4°C. They were held at least a week at 5°C., when it was assumed that acclimation was complete. The lobsters for the 15°C. acclimation were taken in the spring from water which

varied from 7 to 10°C. and were held at 15°C. for two to three weeks. Those for the 25°C. acclimation were caught in late spring when the water temperature ranged from 10 to 11°C. They were held at the surface for two to four weeks where the water gradually warmed to 12 to 14°C. After this they were held for three weeks at 25°C. to complete their acclimation.

After temperature acclimation was complete, at least two weeks were allowed for salinity acclimation. When salinity acclimation was complete, one week was allowed for oxygen acclimation. During salinity and oxygen acclimation, temperature was maintained at the acclimation level. The salinity acclimation levels were maintained during the oxygen acclimation.

The method for determining lethal levels was derived from the constant temperature techniques of Hathaway (1927), Doudoroff (1942), Brett (1944) and Fry *et al.* (1946). For the lobster experiments the level of each of the three factors that kills 50% of the animals with 48 hours' exposure was taken as the lethal level. Fifty per cent lethal levels can be measured precisely whereas the determination of the highest temperature (or lowest salinity or oxygen) that just fails to kill any animals is difficult.

The end point of 48 hours was chosen on the basis of preliminary experiments. These indicated that mortality caused by 48 hours' exposure to high temperature was not significantly less than mortality caused by 72 hours. The same end point was chosen for salinity and oxygen experiments to correspond with the lethal temperature experiments. The suitability of this end point will be discussed in the section on resistance times.

The 50% lethal levels of each of the three factors were derived from the data in similar ways. To illustrate the method, a sample of the lethal temperature results is shown graphically in Fig. 2. The curved line is drawn through points

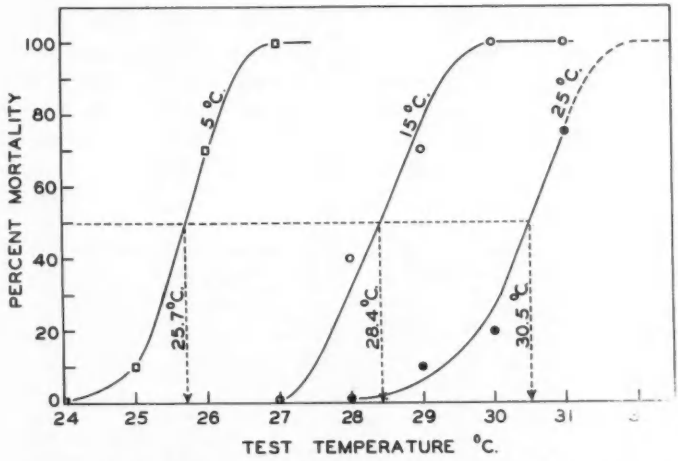

Fig. 2.—Percentage mortality at 48 hours for lobsters acclimated at 5°C., 15°C. and 25°C. each at 30‰ salinity and 6.4 mg. O_2/l. Fifty per cent lethal temperatures are shown on each curve.

representing percentage mortality in the test temperatures at 48 hours. The temperature that would cause 50% mortality can be read directly from the graph. The complete tables from which the lethal levels were determined may be referred to in manuscript form (McLeese, 1954).

Upper Lethal Temperatures

Upper lethal temperatures were determined in four constant temperature tanks maintained at 1°C. intervals with salinity and oxygen maintained at the acclimation level. In each case, the four test temperatures usually caused no mortality at the lowest temperature and 100% at the highest. Ten lobsters were used for each test temperature and mortalities were observed for 48 hours. Observations were taken at hourly intervals during the first 24 hours and every hour and a half or two hours during the second 24 hours. There were minor deviations from this plan when the condition of the lobsters warranted. Temperature, salinity and oxygen levels were determined at the same intervals.

The upper lethal levels of temperature for each of the 27 acclimation combinations are presented in the fourth column of Table VI.

The 27.5°C. lethal temperature for lobsters acclimated to 15°C., 25‰ salinity and 2.9 mg. O_2/l. replaces the original determination of 25.6°C. which is aberrant.

Table VI.—Upper lethal temperature levels and lower lethal salinity and oxygen levels for lobsters acclimated to 27 different combinations of temperature, salinity and oxygen.

Acclimation conditions			Lethal temperature	Lethal salinity	Lethal oxygen
Temp.	Salinity	Oxygen			
°C.	‰	*mg./l.*	°C.	‰	*mg./l.*
		2.9	20.6	11.0	0.72
5	20	4.3	22.0	9.0	0.77
		6.4	23.7	9.0	0.72
		2.9	22.4	12.0	0.57
5	25	4.3	22.1	12.4	0.51
		6.4	24.6	9.2	0.24
		2.9	24.0	10.8	0.29
5	30	4.3	25.2	11.5	0.33
		6.4	25.7	6.0	0.20
		2.9	27.3	9.0	0.86
15	20	4.3	27.7	9.0	0.79
		6.4	27.8	8.2	1.20
		2.9	27.5[a]	10.7	0.80
15	25	4.3	28.2	10.7	0.90
		6.4	28.0	9.5	1.00
		2.9	27.8	10.6	0.66
15	30	4.3	28.2	11.0	0.83
		6.4	28.4	11.2	0.83
		2.9	28.5	11.5	1.72
25	20	4.3	29.0	11.5	1.58
		6.4	29.3	11.1	1.26
		2.9	29.0	14.3	1.17
25	25	4.3	29.5	14.8	1.20
		6.4	29.6	14.0	1.60
		2.9	28.7	15.4	1.30
25	30	4.3	29.5	16.0	1.25
		6.4	30.5	16.4	1.17

[a] Adjusted level, see text.

The low reading is accounted for by irregularities in the 26° and 27°C. tests that were used in its determination. The adjusted value is the average of the lethal levels at 15°C. for 20‰ and 30‰ salinity both at 2.9 mg. O_2/l.

The whole range of lethal temperatures is not presented in the table. At acclimation to temperatures below 5°C. and to salinity and oxygen levels below 20‰ and 2.9 mg. O_2/l., the lethal temperature would be depressed below 20.6°C. The latter conditions, however, are probably going beyond the bounds where temperature alone is operating as a lethal factor into the region where salinity and oxygen may operate as lethal factors.

The lethal levels of temperature listed in Table VI were analysed statistically with the results shown in Table VII.

TABLE VII.—Results from analysis of variance of lethal temperature levels for variously acclimated lobsters. Double asterisks indicate significance at the one per cent level.

Source of variance	Sum of squares	Degrees freedom	Variance
Total	19,603	26	
Temperature acclimation	16,326	2	8163**
Salinity acclimation	737	2	368**
Oxygen acclimation	936	2	468**
Discrepancy	1,004	20	50
Temperature by salinity interaction	443	4	110
Temperature by oxygen interaction	209	4	52
Salinity by oxygen interaction	20	4	5
Error	332	8	41

Temperature, salinity and oxygen acclimation all have a highly significant effect on the lethal temperature level. By reference to Table VI it can be seen that the lethal temperature is raised when the acclimation temperature is raised. It is lowered when the salinity and oxygen acclimation levels are lowered.

LOWER LETHAL SALINITIES

Lower lethal salinities were determined in four constant salinity baths, maintained at 2‰ intervals. The salinity range of 6‰ was chosen to include low salinities that would cause from 0% to 100% mortality with 48 hours' exposure. The lethal levels of salinity for each of the 27 acclimation groups are listed in the fifth column of Table VI. These were analysed statistically with the results shown in Table VIII.

TABLE VIII.—Results from analysis of variance of lethal salinity levels for variously acclimated lobsters. Double asterisks indicate significance at the one per cent level, single at the five per cent level.

Source of variance	Sum of squares	Degrees freedom	Variance
Total	16,334	26	
Temperature acclimation	8,847	2	4423**
Salinity acclimation	2,682	2	1341**
Oxygen acclimation	906	2	453*
Discrepancy	3,899	20	195
Temperature by salinity interaction	1,877	4	469*
Temperature by oxygen interaction	1,052	4	263
Salinity by oxygen interaction	204	4	51
Error	766	8	96

Temperature and salinity acclimations have highly significant effects on the lethal salinity level. In addition, oxygen acclimation and a temperature by salinity interaction are significant.

The directions of these effects can be seen by reference to Table VI. The lethal salinity level is raised by an increase in the level of temperature acclimation and by a decrease in the level of oxygen acclimation. A decrease in the level of salinity acclimation lowers the lethal salinity level. The significant interaction between temperature and salinity means that the effect of salinity acclimation is not always the same, but depends on the temperature at which lobsters are acclimated.

Lower Lethal Oxygen Levels

Lower lethal oxygen levels were determined in four constant oxygen baths maintained at intervals of approximately 0.3 mg. O_2/l. The range of oxygen, about 1.0 mg. O_2/l., was chosen to include low oxygens which would cause from 0% to 100% mortality with 48 hours' exposure.

The lethal oxygen levels for each of the 27 acclimation groups are listed in the sixth column of Table VI. These were analysed statistically with the results listed in Table IX.

Table IX.—Results from analysis of variance of lethal oxygen levels for variously acclimated lobsters. Double asterisks indicate significance at the one per cent level.

Source of variance	Sum of squares	Degrees freedom	Variance
Total	22,005	26	
Temperature acclimation	16,989	2	8494**
Salinity acclimation	2,809	2	1404**
Oxygen acclimation	74	2	36
Discrepancy	2,133	20	107
Temperature by salinity interaction	147	4	36
Temperature by oxygen interaction	679	4	170
Salinity by oxygen interaction	153	4	38
Error	1,154	8	144

Oxygen acclimation does not have a significant effect on the lethal oxygen level. Temperature and salinity acclimation both have a highly significant effect on the lethal oxygen level. By reference to Table VI, it can be seen that the lethal oxygen level is raised by an increase in the level of temperature acclimation and by a decrease in the level of salinity acclimation.

Unless precautions are taken there is danger of carbon dioxide accumulation in experiments involving low levels of oxygen. This was avoided in the present experiment by driving off excess CO_2 in acclimation, lethal temperature and salinity tanks, using the method of Thomas (1954). For lethal oxygen tests, low levels of oxygen were obtained by passing the water through a nitrogen atmosphere, which prevents increase in CO_2 content.

Lower Lethal Temperatures

Resistance to cold is lost to some extent as animals show a gain in heat tolerance with acclimation to high temperature (Fry *et al.*, 1946). Approximate

lower lethal temperatures have been established for lobsters acclimated at 17.0° and 27.5°C. The results are presented in Table X.

All animals died within 24 hours in the 17.0°C. acclimation group. In upper lethal experiments, temperatures that killed all the animals in 24 hours were about 1.5°C. higher than those which killed half the lobsters in 48 hours. Using 1.5°C. as a correction factor, the lower lethal temperature for 17.0°C. acclimated

TABLE X.—Mortalities of lobsters acclimated to 17.0° and 27.5° C., when tested at low temperatures.

Acclimation temperature	Test temperature	Number of specimens	Number dead at stated hours						
			1	3	6	18	24	48	72
°C.	°C.								
17.0	0.3	7	0	0	0	6	7		
27.5	4.8	10	0	1	2	4	5	6	6
27.5	6.0	7	0	0	0	0	0	0	0

lobsters was estimated to be 1.8°C. The 50% lethal temperature in 48 hours for lobsters acclimated to 27.5°C. was estimated to be 5°C. Although these lower lethal temperatures are estimates, they indicate the approximate lower limit of thermal tolerance.

RESISTANCE TIMES

An animal can withstand exposure to levels of an environmental factor within the lethal zone provided the exposure does not exceed certain finite periods which depend on the level of the factor. The time taken to cause 50% mortality at a lethal level of the factor is usually taken as the resistance time. When the logarithm of resistance times in a series of lethal levels of the factor is plotted against the level of the factor, straight lines can be drawn through the resistance times.

Fry *et al.* (1946) discovered a break in the slope of lines relating median resistance times and upper lethal temperatures. The temperature at which the break occurred was just below the level at which just 50% mortality occurred (the "incipient lethal level"). At temperatures below this, median resistance times rapidly approached infinity. As Brett (1952) points out, the discovery of this break allows the selection of the proper duration of experiments if precise measurement of the incipient lethal level is desired.

Some of the data for the lobster lethal experiments have been plotted in a similar fashion to determine the relationship between the 48-hour lethal level obtained from the experiments and the incipient lethal levels (McLeese, 1954). As an example, thermal resistance lines for lobsters acclimated to three levels of temperature (5°, 15° and 25°C.) each at three levels of salinity (20, 25 and 30‰) and one level of oxygen (6.4 mg./l.) are shown in Fig. 3, together with the corresponding lethal levels of temperature from Table VI. The lethal levels can be used here since by definition they cause 50% mortality in 48 hours (2880 minutes).

Two of the resistance lines at 5°C., and probably the third, appear to have breaks after which the slope becomes zero. For these, longer exposure to the experimental conditions would not lower the lethal level because the slope of the lines is zero and the observed lethal level probably approximates the true incipient lethal level. The lines at 15° and 25°C., however, do not have breaks by 48 hours and exposure for longer times would possibly lower the lethal level.

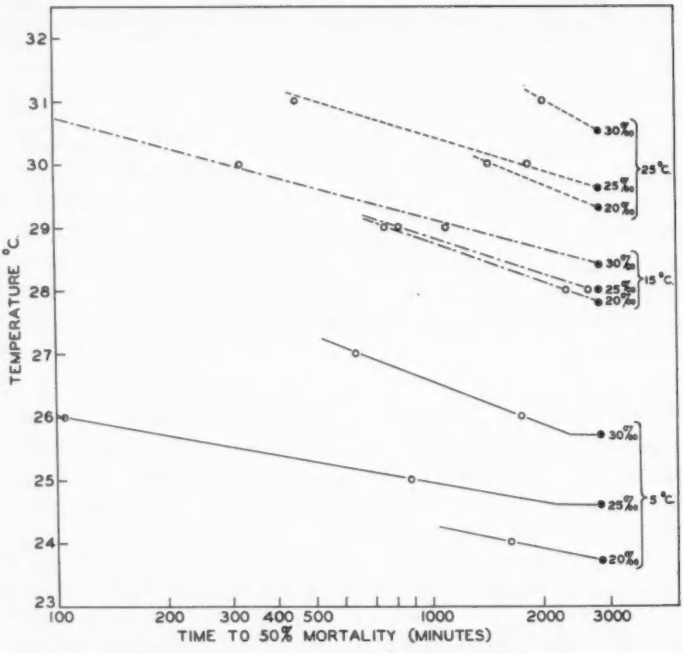

FIG. 3.—Temperature resistance at various lethal levels of temperature for lobsters acclimated to 5, 15 and 25°C. each at three levels of salinity (20, 25 and 30‰) at 6.4 mg. O_2/l. Black circles are lethal levels from Table VI.

A similar situation was found with salinity and oxygen resistance lines. Incipient lethal levels were obtained for some of the acclimation groups but not all.

The results of the experiments do not apply to periods longer than 48 hours since incipient lethal levels with the understood indefinite exposure times were not obtained in all cases.

Ultimate, Maximum and Minimum Lethal Levels

To determine the extremes of temperature, salinity and oxygen levels which can be tolerated by the species, the ultimate and maximum lethal temperature levels and the minimum lethal levels for salinity and oxygen that could be attained through acclimation were estimated.

Fry *et al.* (1942) defined the *ultimate upper incipient lethal temperature* as the temperature beyond which no increase in the lethal temperature results from further increase in acclimation temperature. They were working with a freshwater fish which was not exposed to varying levels of salinity. For the lobster, acclimation to reduced levels of salinity and oxygen both lower the lethal level of temperature. Under such conditions the ultimate lethal temperature cannot be attained but the lethal temperature does reach a slightly lower maximum value depending on the salinity and oxygen conditions. These are termed *maximum lethal levels* in this paper. Similarly, for lower lethal salinity and oxygen levels, the *minimum lethal level* is used as the name of the lowest lethal in cases where the ultimate lower lethal level cannot be attained.

TEMPERATURE. Fry *et al.* (1942) developed a method for describing the thermal tolerance of fish as an area instead of expressing it in terms of points. The ultimate upper lethal temperature can be determined from this method of plotting thermal tolerance.

The tolerance area is bounded above and below by lines relating the upper and lower lethal temperature to the acclimation temperature; it is bounded laterally by two perpendicular lines, one erected at 0°C. acclimation and the other at that acclimation temperature which is equal to the ultimate upper lethal temperature.

In their work with freshwater animals, Fry *et al.* (1942) did not study the tolerance of animals below 0°C., the freezing point of fresh water. Similarly, it is not practical to study the tolerance of marine species below the freezing point of sea water. The main interest in a tolerance diagram for the lobster in this paper is its contribution to the determination of the highest levels to which the upper lethal temperature can be raised for the different acclimation groups. For this reason, no attempt has been made to project the tolerance diagram beyond 0°C.

Figure 4 illustrates the data for lobsters acclimated at 30‰ salinity. The lower lethal temperatures were taken from Table VII. The ultimate and maximum lethal temperature levels are indicated by arrows.

Similar diagrams have been drawn for lobsters acclimated at 25‰ and 20‰ salinity. They are not reproduced here but the maximum lethal temperatures derived from them are shown in Table XI.

The area within the tolerance diagram (tolerance zone) can be expressed numerically as degrees centigrade squared and is a relative measure of the tolerance of the animal. The tolerance zone for lobsters acclimated to 30‰ salinity and 6.4 mg. O_2/l. has an area of 830 in this unit. Areas for four fishes are computed by Brett (1944) and Fry *et al.* (1946) as follows:

Species	Tolerance	Original data
Goldfish (*Carassius auratus*)	1220	Fry *et al.*, 1942
Bullhead (*Ameiurus nebulosus*)	1162	Brett, 1944
Greenfish (*Girella nigricans*)	800	Doudoroff, 1942
Speckled trout (*Salvelinus fontinalis*)	625	Fry *et al.*, 1946
Lobster	830	This paper

The thermal tolerance of the lobster, in this area unit, is intermediate among

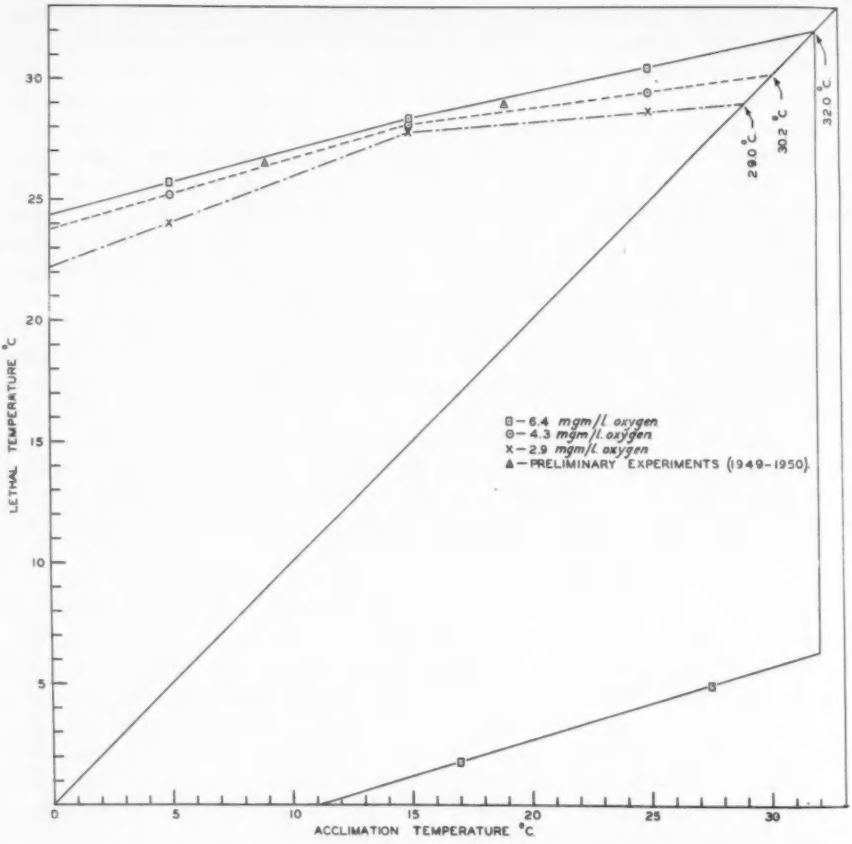

FIG. 4.—Thermal tolerance of lobsters acclimated to 30‰ salinity and 2.9, 4.3 and 6.4 mg. O_2/l. Arrows show ultimate and maximum lethal temperatures.

those for freshwater fishes, and is approximately equal to that of the marine greenfish.

Comparisons of the numerical values for tolerance areas can be misleading because they do not describe the shape of the diagram or the relationships between acclimation and lethal levels.

The zone of tolerance as shown for the lobsters includes an area that is lethal to 50% of the animals in 48 hours by virtue of its derivation from lethal levels. The zone in which no animals will die is somewhat smaller.

SALINITY. The lethal salinity data can be plotted as salinity tolerance diagrams similar to that for temperature although there are some major differences between the two. In a marine environment, reduced rather than increased salinity is most likely to operate as a lethal factor. Therefore, upper lethal salinity levels are not

shown on the tolerance diagram. As well, the data are somewhat restricted because acclimations between 20‰ and 30‰ only were studied. Difficulties are experienced in acclimating lobsters to salinities much below 20‰, especially at reduced levels of oxygen and high temperatures. Salinities much above 30‰ cannot be obtained unless the salinity is artificially increased.

The tolerance diagram for 15°C. lobsters is shown in Fig. 5. If the lethal lines are projected back to meet the diagonal construction line, they meet the line between 2 and 3‰ salinity. Lobsters cannot be acclimated to these low salinities which are lethal and the projected line must have a point of inflection at which the line becomes parallel to the abscissa. Further acclimation to reduced salinity beyond the point of inflection would not alter the lethal level. Since the actual inflection points are not known, the lethal level for lobsters acclimated to 20‰ salinity has arbitrarily been chosen as the inflection point. In other words, it is considered that the lethal levels for 20‰ salinity acclimation are the minimum lethal levels.

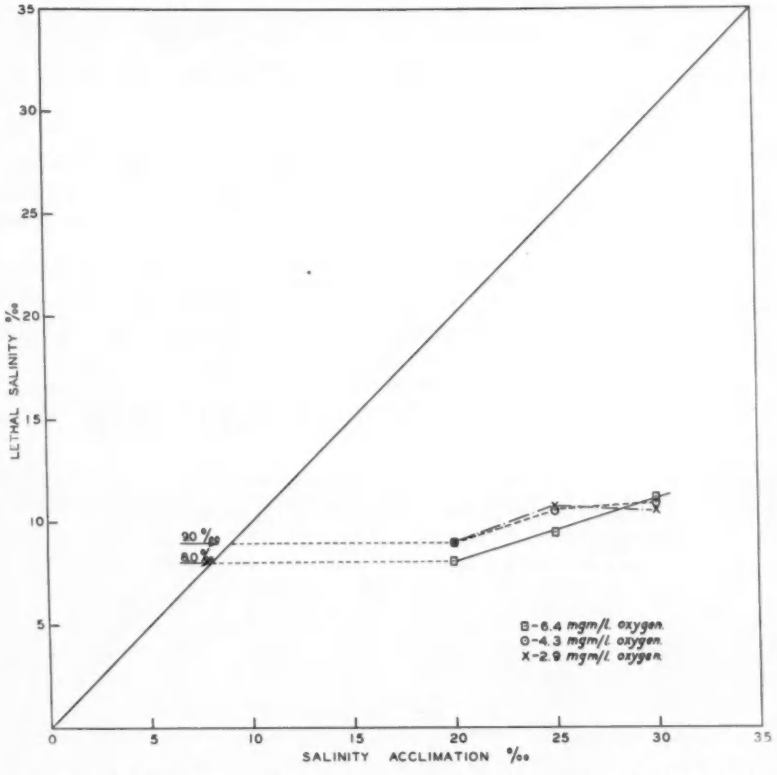

FIG. 5.—Salinity tolerance of lobsters acclimated to 15°C. and 2.9, 4.3 and 6.4 mg. O_2/l. Arrows show minimum lethal levels.

The figures for 5° and 25°C. acclimation are not reproduced since the minimum lethal salinities can be obtained directly from the 20‰ acclimations in Table VI.

OXYGEN. The lethal oxygen results are presented as tolerance diagrams in Fig. 6. The slopes of the lethal lines are not consistent in direction and statistical analysis of the data has shown that oxygen acclimation does not have a significant

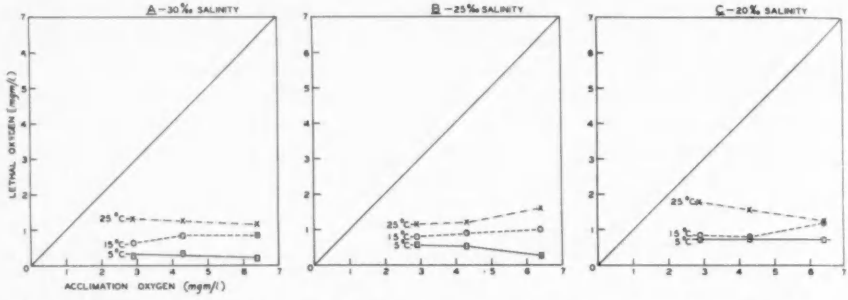

FIG. 6.—Oxygen tolerance of lobsters acclimated to 5°, 15° and 25°C. each at 2.9, 4.3 and 6.4 mg. O_2/l.

effect on the lethal oxygen levels. Therefore, the points of intersection where the lethal lines meet the diagonal construction line will not give satisfactory minimum lethal oxygen levels. In this case, the best estimate of these levels is obtained by using the average of the data contributing to each line.

Oxygen acclimation was shown to exert a significant effect on the lethal temperature and salinity levels, but not on the lethal oxygen level. It is possible that the effect in the two former cases was not an acclimation effect but simply the result of the low levels of oxygen prevailing at the time of the tests.

In summary, the maximum lethal temperatures and the minimum lethal salinity and oxygen levels have been assembled in Table XI. The only "ultimate"

TABLE XI.—Maximum upper lethal levels of temperature, and minimum lower lethal levels of salinity and of oxygen, for lobsters acclimated to 27 combinations of temperature, salinity and oxygen. The temperature indicated by an asterisk is the *ultimate* lethal level.

Temperature			Salinity			Oxygen		
Acclimation		Ultimate and	Acclimation		Minimum	Acclimation		Minimum
Salinity	Oxygen	maximum levels	Temp.	Oxygen	level	Temp.	Salinity	level
‰	*mg./l.*	° C.	° C.	*mg./l.*	‰	° C.	‰	*mg./l.*
30	6.4	32.0*	25	6.4	11.2	25	30	1.24
30	4.3	30.2	25	4.3	11.5	25	25	1.32
30	2.9	29.0	25	2.9	11.7	25	20	1.52
25	6.4	30.5	15	6.4	8.0	15	30	0.77
25	4.3	30.1	15	4.3	9.0	15	25	0.90
25	2.9	29.8	15	2.9	9.0	15	20	0.95
20	6.4	30.1	5	6.4	9.0	5	30	0.27
20	4.3	29.6	5	4.3	11.5	5	25	0.44
20	2.9	29.0	5	2.9	11.5	5	20	0.74

level which could be estimated was that for temperature, which is 32.0°C. under optimum conditions of 30‰ salinity and 6.4 mg./l. of oxygen.

BOUNDARY OF LETHAL CONDITIONS

A three-dimensional diagram relating the lethal levels of the three factors has been prepared (Fig. 7) using the ultimate, maximum and minimum lethal levels shown in Table XI.

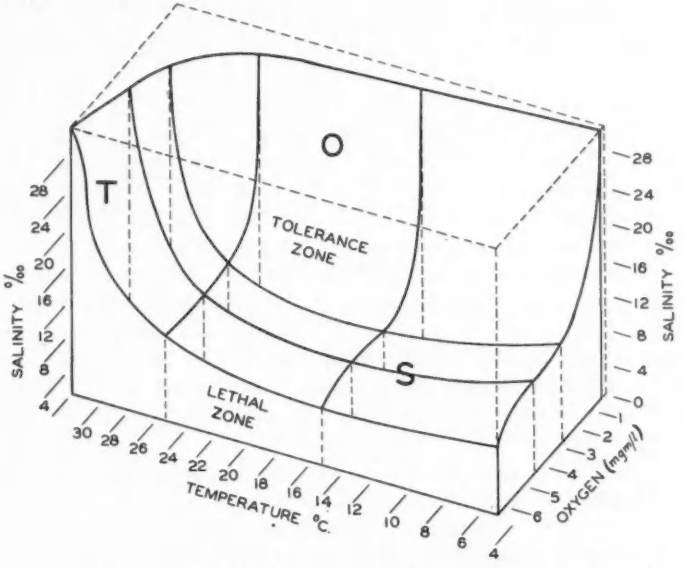

FIG. 7.—Diagram of the boundary of lethal conditions for lobsters for various combinations of temperature, salinity and oxygen.
T—region in which temperature alone acts as a lethal factor
S—region in which salinity alone acts as a lethal factor
O—region in which oxygen alone acts as a lethal factor

Temperature and oxygen were plotted against each other on the horizontal plane and provided the base for the model. Templates, scaled to represent the minimum lethal salinities were erected on this base. In all, six templates were erected, three on the oxygen lines at 2.9, 4.3 and 6.4 mg. O_2/l. and three on the temperature lines at 5, 15 and 25°C. The smooth curves of the templates form the boundary between the lethal zone below and the tolerance zone above.

The diagram defines a segment of the complete zone of tolerance for the lobster to combinations of temperature, salinity and oxygen. It is bounded on the top and on the front by the salinity and oxygen contents of sea water, respectively. Experiments with concentrated sea water and supersaturated oxygen content would be necessary to extend the scope of the diagram into these regions. The diagram could also be projected toward the right to the freezing point of sea water.

The diagram provides a method for assessing the lethal effects of any one of temperature, salinity or oxygen with respect to the other two factors. The steep slope of the boundary surface from 25°C. up to 32°C. and from approximately 1.4 mg. O_2/l. to lower oxygen values is perhaps the most striking feature of the diagram. This indicates that high temperature and low oxygen each affect the survival of the lobster within a limited range only. The flat areas in the diagram are areas where one of the factors only is operating as a lethal factor. The flat area S, for oxygen values between 6.4 and 2.9 mg. O_2/l., at temperatures between 5° and 22°C., probably represents an area where temperature and oxygen conditions exert little stress on the organism and death is primarily due to low salinity. A similar situation prevails for oxygen values of 1.1 mg. O_2/l. and less between 5 and 21°C. (area O). Here the salinity values are between 16 and 30‰ and death is caused by low oxygen. The area T, for oxygen values between 6.4 and 2.9 mgm. O_2/l., at temperatures between 28 and 30°C., probably represents an area where high temperature is the lethal agent.

The area where the temperature and oxygen surfaces meet depicts an area of mixed lethal effects of high temperature and low oxygen. In addition, there is a mixed lethal effect of temperature and salinity at approximately 25°C. at high levels of oxygen, and a mixed lethal effect of salinity and oxygen at approximately 2.9 mg. O_2/l. at low temperatures. The picture of mixed lethal effects is further complicated in the region where the areas T, S and O come together. This is an area where temperature, salinity and oxygen all combine to exert a lethal effect.

The diagram was constructed from ultimate, maximum and minimum lethal levels and as such defines the zone of tolerance for lobsters fully acclimated to the conditions of the experiment. The tolerance of lobsters not fully acclimated to the experimental conditions would be somewhat less than shown by the diagram. In addition, the surface is based on 50% lethal levels and therefore actually includes a small part of the lethal range.

The information shown in Fig. 7 can be recorded in a more convenient tabular form. The salinities at which half the lobsters will die in 48 hours have been read from the model for particular combinations of temperature and oxygen and are listed in Table XII.

TABLE XII.—Lethal salinities of lobsters for various combinations of temperature and oxygen. When lobsters are *gradually* subjected to each of the combinations indicated, their average mortality is 50 per cent.

	Temperature ° C.						
Oxygen	5	9	13	17	21	25	29
mg./l.	Salinity, ‰						
1.0	18.0	21.0	20.4	22.0	30.0	...	...
2.0	13.3	12.6	12.0	11.2	12.0	15.0	...
3.0	11.6	10.4	9.6	9.0	9.3	11.2	30.0
4.0	11.4	9.7	9.4	9.0	9.3	11.2	20.4
5.0	11.0	9.5	8.8	8.8	9.3	11.2	17.8
6.0	9.8	8.8	8.4	8.4	9.3	11.2	16.4

The table is useful in determining the cause of death of lobsters in commercial holding units. Such information is of value in providing a basis for advice toward the improvement of holding conditions.

A complete description of the lethal effects of an environmental factor requires a statement of the acclimation histories of the animals since the lethal level may vary with acclimation. Where three factors of the environment are at extreme levels at the same time, the lethal levels for each factor are related to the various acclimation levels of all three factors. It is impossible, therefore, to depict the combined lethal effects of the three factors using the data in their original form. The problem has been resolved by deriving ultimate and maximum or minimum lethal levels. At these points the acclimation levels are equal to the lethal levels. The acclimation levels can, therefore, be eliminated from a graphic presentation and the combined lethal effects of the three factors can be illustrated in a single figure. Such an illustration also represents the extreme conditions to which the animal can be acclimated.

REFERENCES

Bělehrádek, J. 1935. Temperature and living matter. *Protoplasma-Monographien*, Vol. 8. Gebrüder Borntraeger, Berlin, 229 pp.

Bert, P. 1871. Sur les phénomènes et les causes de la mort des animaux d'eau douce que l'on plonge dans l'eau de mer. *C. R. Acad. Sci. (Paris)*, **73**: 382–385.

Brett, J. R. 1944. Some lethal temperature relations of Algonquin Park fishes. *Univ. Toronto Stud., Biol. Ser.*, No. 52; *Pub. Ontario Fish. Res. Lab.*, No. 63, 49 pp.

1946. Rate of gain of heat-tolerance in goldfish (*Carassius auratus*). *Ibid.*, No. 53; *Pub. Ontario Fish. Res. Lab.*, No. 64, 28 pp.

1952. Temperature tolerance in young Pacific salmon, Genus *Oncorhynchus*. *J. Fish. Res. Bd. Canada*, **9**: 265–323.

Chaisson, A. F. 1931. The effect of low salinities on the lobster. *Ann. Rep. Biol. Bd. Canada* for 1931, pp. 1–90.

1932. Factors in shipping of live lobsters from Eastern Nova Scotia. *Bull. Biol. Bd. Canada*, No. 33: 3–29.

Donahue, J. K. 1953. Studies on ecdysis in the American lobster (*Homarus americanus*). 2. Serum calcium levels under various normal conditions. *Maine Dept. Sea and Shore Fish., Res. Bull.* No. 13, pp. 3–11.

Doudoroff, P. 1942. The resistance and acclimatization of marine fishes to temperature changes. I. Experiments with *Girella nigricans* (Ayres). *Biol. Bull.*, **83**: 219–244.

Fry, F. E. J. 1947. Effects of the environment on animal activity. *Univ. Toronto Stud., Biol. Ser.*, No. 55; *Pub. Ontario Fish. Res. Lab.*, 68, 62 pp.

1951. A fractionating column to provide water of various dissolved oxygen content. *Canadian J. Technol.*, **29**: 144–146.

Fry, F. E. J., J. R. Brett and G. H. Clawson. 1942. Lethal limits of temperature for young goldfish. *Rev. Canadienne de Biol.*, **1**: 50–56.

Fry, F. E. J., J. S. Hart and K. F. Walker. 1946. Lethal temperature relations for a sample of young speckled trout (*Salvelinus fontinalis*). *Univ. Toronto Stud., Biol. Ser.*, No. 54; *Pub. Ontario Fish. Res. Lab.*, No. 66, 35 pp.

Hart, J. S. 1952. Geographic variations of some physiological and morphological characters in certain fresh-water fish. *Univ. Toronto Stud., Biol. Ser.*, No. 60; *Pub. Ontario Fish. Res. Lab.*, No. 72, 79 pp.

HATHWAY, E. S. 1927. Quantitative study of the changes produced by acclimatization on the tolerance of high temperatures by fishes and amphibians. *Bull. U. S. Bur. Fisheries*, **43**: 169–192.

HOAR, W. S., AND J. E. DORCHESTER. 1949. The effect of dietary fat on the heat tolerance of goldfish (*Carassius auratus*). *Canadian J. Res.*, D, **27**: 85–91.

HOLLETT, A. 1943. The relation between moult cycle and the phosphorous content of the blood and muscle in lobster. *J. Fish. Res. Bd. Canada*, **6**: 152–157.

HUNTSMAN, A. G., AND M. I. SPARKS. 1924. Limiting factors for marine animals. 3. Relative resistance to high temperature. *Contr. Canadian Biol.*, N. S. **2**(6): 97–114.

KNUDSEN, M. 1946. The determination of the chlorinity of sea water and hydrographic tables. Woods Hole Oceanographic Inst., Woods Hole, Mass.

LOEB, J., AND G. WASTENEYS. 1912. On the adaptation of fish (*Fundulus*) to higher temperature. *J. Exp. Zool.*, **12**: 543–557.

LOOSANOFF, V. L. 1945. Effects of sea water of reduced salinity on starfish *A. forbesi* of Long Island Sound. *Trans. Connecticut Acad. Arts and Sci.*, **36**: 813–833.

LOOSANOFF, V. L., AND P. B. SMITH. 1949. Some aspects of behaviour of oysters accustomed to different salinities. *Anat. Record*, **105**(3): 147.

LOWNDES, A. G., AND N. K. PANIKKAR. 1941. A note on the changes in water content of the lobster, (*Homarus vulgaris* M.-Edw.) during moult. *J. Mar. Biol. Assoc. U. K.*, N. S. **25**: 111–112.

MCLEESE, D. W. MS, 1950. Factors limiting the survival of lobsters. *Fish. Res. Bd. Canada, MS. Rep. Biol. Sta.*, No. 406, 10 pp.

MS, 1954. Effects of temperature, salinity and oxygen on the survival and distribution of the lobster (*Homarus americanus* Milne-Edwards). *Fish. Res. Bd. Canada, MS. Rep. Biol. Sta.*, No. 589, 62 pp.

NIKIFOROF, N. D. 1953. [Variation in intensity of respiration in young salmon, reared under different conditions of oxygen regime.] *Doklady Akad. Nauk S.S.S.R.*, **88**: 165–167.

PANIKKAR, N. K. 1941. Osmoregulation in some palaemonid prawns. *J. Mar. Biol. Assoc. U.K.*, N.S. **25**: 317–359.

QUIGLEY, J. P. 1928. Reaction of an Elasmobranch (*Squalus suckleyi*) to variations in the salinity of the surrounding medium. *Biol. Bull.*, **54**: 165–190.

SHEPARD, M. P. 1955. Resistance and tolerance of speckled trout, *Salvelinus fontinalis*, to oxygen lack. *J. Fish. Res. Bd. Canada*, **12**: 387–433.

SMITH, G. F. M. MS, 1951. The physiology of thermal and salinity tolerance in lobsters. Report to the Associate Committee on Research in Aquatic Biology, National Research Council, Canada, 5 pp.

SUMNER, F. B., AND P. DOUDOROFF. 1938. Some experiments on the temperature acclimatization and respiratory metabolism in fishes. *Biol. Bull.*, **74**: 403–429.

TEMPLEMAN, W. 1936a. Local differences in the life history of the lobster, *Homarus americanus*, on the coast of the Maritime Provinces of Canada. *J. Biol. Bd. Canada*, **2**: 41–88.

1936b. The influence of temperature, salinity, light and food conditions on the survival and growth of the larvae of the lobster *Homarus americanus*. *Ibid.*, **2**: 485–497.

1944. Abdominal width and sexual maturity of female lobsters on Canadian Atlantic Coast. *J. Fish. Res. Bd. Canada*, **6**: 281–290.

THOMAS, H. J. 1954. The oxygen uptake of the lobster (*Homarus vulgaris* Edw.). *J. Exp. Biol.*, **31**: 228–251.

TOPPING, F. L., AND J. L. FULLER. 1942. The accommodation of some marine invertebrates to reduced osmotic pressure. *Biol. Bull.*, **82**: 372–384.

WILDER, D. G. 1953. The growth rate of the American lobster (*Homarus americanus*). *J. Fish. Res. Bd. Canada*, **10**: 371–412.

WOOD, W. M. 1885. Transplanting lobsters to the Chesapeake–Experiments upon the temperature which they can endure. *Bull. U.S. Fish Comm.* **5**: 31–32.